Food Research

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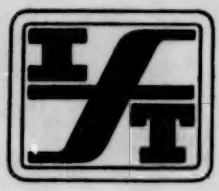

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WHY SHOULD THE VITAMIN C CONTENT OF FRUIT AND VEGETABLE JUICES BE STANDARDIZED?

Because nutrition reports disclose that—

- **Vitamin C deficiencies exist throughout the country***

Proof of deficiencies among adults and children. You should know these facts!* Diet surveys from Maine and Vermont, New York, Illinois, New Mexico, Oregon, West Virginia, and Texas show a definite pattern of inadequate vitamin C intake. These studies covered grade school and high school children, college men and women.

Spongy, swollen gums symptomatic of scurvy.

From these studies* of dietary habits and physical conditions there is considerable proof that many of the children and adults covered by the surveys have actual, physical symptoms of vitamin C deficiency.

Infantile scurvy. Scurvy* occurs, even today, among infants who are artificially fed. Fortunately the disease may be cured by the administration of vitamin C. How much more sensible it is to prevent it by supplying these bottle-fed infants with the daily allowance of 30 milligrams of vitamin C as recommended by the Food and Nutrition Board of the National Research Council for children under one year of age.

Symptoms of infantile scurvy are fretfulness and fear of being touched.

These bottle-fed infants, unless receiving a pediatric prescription containing vitamin C, are largely dependent on supplemental ascorbic acid which they receive from fruit or vegetable juices. Unless fortified with vitamin C many of the processed juices may not supply these babies with adequate quantities of this essential element.

- **Scurvy has not disappeared from the United States***

Typical position of legs in infantile scurvy.

What can be done? Because of the increasing evidence that American diets are deficient in vitamin C, leading nutrition experts believe that processed fruit and vegetable juices, on which the public is so reliant for its supply of this necessary vitamin, should contain the "Vital Ingredient."

The Vital Ingredient of any processed fruit or vegetable juice is a *dependable* and *adequate* supply of vitamin C. You can give your juice this ingredient by *standardizing its vitamin C content.* The processing is simple and the new low cost of ascorbic acid makes juice standardization more attractive than ever.

With ascorbic acid available *by the tons* at extremely modest cost fruit and vegetable juices can have a standardized vitamin C content for the better protection of the nation's health and well-being.

*Statements are based on documented reports. References on request.

Illustrations after photographs in The Vitamins in Medicine by Bicknell and Prescott, Third Edition, published by Grune & Stratton, Inc., New York.

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NUTRIENT COMPOSITION OF CUBAN FOODS[a,b]
I. FOODS OF VEGETABLE ORIGIN

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(Manuscript received November 6, 1954)

All countries of the world have serious problems in feeding their people, and Cuba is no exception. In order to develop a sound food and nutritional program in any country, it is necessary first to conduct surveys of (a) food resources, (b) food composition, (c) diet composition, and (d) the clinical status of the people. When the results of these surveys are at hand, nutritionists and educators are in a position to improve intelligently the nutritional status of the people.

Surveys of the edible flora and fauna of Cuba, and of the nutritive composition of these foods was started in 1952, as an initial step in providing the data that are required.

The scientific literature indicates *(1, 3, 5-8, 12, 13, 25, 31, 32)* that several Cuban investigators have analyzed a few Cuban foods, but these reports are fragmentary and incomplete.

This is a report of the analysis of 115 samples of foods of vegetable origin collected in Cuba between February and December 1953. The following nutrients were determined in each sample: moisture, ether extract, crude fiber, nitrogen, ash, calcium, phosphorus, iron, carotene, thiamine, riboflavin, niacin, ascorbic acid, tryptophane, methionine, and lysine.

EXPERIMENTAL

Collection of samples. In this program it was planned to survey representative foods collected in various parts of Cuba. The country was divided into 7 primary and 15 secondary agricultural zones. For convenience each secondary zone was centered in a principal market town (Fig. 1). The collection team traveled throughout Cuba in a Jeep station wagon, carrying with it the supplies and equipment needed for the collection of specimens and the stabilization of samples. Whenever possible, the samples were taken directly from the fields in order to minimize losses in food values. It was occasionally necessary to collect samples in market-places, but in each instance an accurate history of the sample was obtained.

[a] This investigation has been possible through the initiative and support of the Comité Cubano-Americana Pro Fundación de Investigaciones Médicas and with the cooperation of the Cuban Government, the Williams-Waterman Fund, the Instituto Finlay, the Esso Standard Oil Company and the United Fruit Company.

[b] Publication No. 1, Fundación de Investigaciones Médicas, No. 14 Journal Series, Atkins Garden & Research Laboratories, Harvard University, and No. 253 Department of Food Technology, M.I.T.

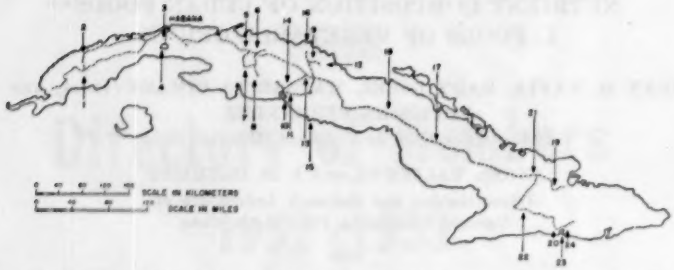

Figure 1. Showing origin of samples in Cuban Food Survey and Survey travel (1953)

Legend:

1. Pinar del Río
2. Viñales
3. La Salud
4. Güira de Melena
5. Agramonte
6. Colón
7. Jagüey Grande
8. Manguito
9. Calabazar de Sagua
10. Cienfuegos
11. Soledad
12. Encrucijada
13. Esperanza
14. Palmira
15. Trinidad
16. Sagua la Grande
17. Camagüey
18. Ciego de Avila
19. Cueto
20. El Cobre
21. Holguín
22. Jiguani
23. Santiago de Cuba
24. Caney

Within a few hours of collection, all food materials were slurried in a Waring blender, stabilized, sealed in wide-mouthed 500-ml. jars, and refrigerated until shipped to the laboratory. All shipping boxes reached the laboratory within 12 hours.

Dry seeds were shipped in sealed jars without further preparation. In the preparation of the remaining samples 3 slurries of each were made *(8, 17)*. Subsample A was prepared by blending 100 g. of the edible portion with 100 ml. of alkaline alcohol solution (30 g. KOH, 320 g. 95% ethanol, and water to make 1000 ml.). Subsample B was prepared from 400 g. of food slurried with 200 or 400 ml. of 1% oxalic acid solution, the amount used was dependent on the water content of the sample. Subsample C was prepared by slurrying 50 g. of food with 200 ml. of 1% oxalic acid solution. Just before blending was completed, 10 and 5 ml. of chloroform were added to subsample B and C, respectively. Subsample A was used for carotene analysis in the laboratory, subsample C for ascorbic acid analysis, and subsample B for all the remaining analyses.

Pertinent information on each sample was recorded on forms which provided space for data on nomenclature, agricultural practices, climate, soil, methods of preparation, use of the material as food in the zone of origin, description of the procedures used in field sampling, and in preparing the slurries. A 35 mm. Kodachrome was made of each sample as a permanent record of its appearance. Herbarium specimens and propagating material were often taken to assist in the precise identification of samples.

Climatological data are seldom available in Cuba, and rainfall gauges quite rarely found except at airfields and sugar estates. It is estimated that the probable rainfall in the areas visited in this study is in the range of 45 to 65 inches per year. In all collection areas, a well-defined monsoon climate exists, with a clearly marked hot rainy season and a cool dry season. Different rainfall conditions exist in other parts of Cuba, and samples will be taken in these areas in subsequent studies. In no case were samples taken at altitudes above 1500 feet; practically all of Cuba's field crops are produced below this level.

Very little detailed information on Cuban soils is available, although a general reconnaissance study and a map were made a number of years ago *(4)*. Because of this, soils data are recorded mostly in the form of physical descriptions, and estimates of fertility are made on the basis of the native vegetation and the condition of the crops. Because of the dearth of habitat information, which might prove to have bearing on the variation in nutrient values, the geographical origin of each collection

taken in the field has been recorded with considerable precision (Figure 1) with the expectation that eventually climate and soils studies will permit the insertion of more exact data. Such precise recording of origins is also of value to the plant breeder who wishes to test, increase, and distribute varieties of food plants which this study shows to be of high food value.

Analytical methods. The methods used in this investigation were generally the same as those used by Munsell *et al. (17-24)*. This will permit a direct comparison between the values obtained in Cuba with those obtained in Central America. All analyses were done in duplicate.

Moisture, ether extract, crude fiber, ash, and calcium were determined according to methods described in the A.O.A.C. *(2)*, and nitrogen by the method of Hamilton and Simpson *(11)*. Iron was measured by the α,α-dipridyl procedure described by Hahn *(10)*, and phosphorus by the modification suggested by Lowry and López *(14)*. A chromatographic method, based on the work of Moore *(16)* and modified later by Wall and Kelley *(33)*, was used to determine carotene.

The values reported as "carotene" included α- and β-carotene, and the neo-isomers of carotene present in the samples. Ascorbic acid was determined always within 48 hours after samples were received, using the method of Roe and Oesterling *(26)*. Thiamine was measured by the thiochrome method, and riboflavin by the fluorometric method as described by Munsell *et al. (17)*.

Niacin was measured by the microbiological method described in the U. S. Pharmacopoeia XII *(30)* using *Lactobacillus plantarum* 17-5 as the test organism. In order to evaluate the quality of the protein in the samples three of the most critical essential amino acids (tryptophan, methionine, and lysine) were determined by the microbiological technique, using *Lactobacillus plantarum* 17-5 for tryptophan, and *Leuconostoc mesenteroides* P-60 for methionine and lysine as test organisms. Tryptophan was freed from the food samples by the Greene-Black *(9)* and Schweigert-Snell *(27)* techniques using alkaline hydrolysis and autoclaving the samples with 20 ml. of 5N NaOH for 10 hours at 15 pounds pressure with the addition of 200 mg. of cysteine. Methionine and lysine were hydrolyzed from the food proteins by autoclaving with 25 ml. of 3N HCl for 16 hours at 15 pounds pressure, according to Schweigert *et al. (28)*. Methionine and lysine were analyzed according to Steele *et al. (29)*. In the methionine assay the amino acids in the culture medium were replaced by hydrogen peroxide-treated peptone, with the addition of cysteine, tyrosine and tryptophan as described by Lyman *et al. (15)*.

DESCRIPTION AND USE OF THE FOODS STUDIED

The following is a description of the foods, the compositions of which are presented in Table 1.

CEREALS

Corn: *(Zea mays)*. Corn (maíz) was an established field crop of the Taino Indians of Cuba when Columbus arrived, and the cereal retains its important place in Cuban agriculture, although consumption is far below that of rice. It is eaten as a vegetable while in the milk or dough stages, as corn on the cob in the later dough stage, as fried cakes after scraping from the cob, and as corn meal made from the parched grains.

The samples of "maíz tierno" or green corn collected were in the dough (a) and in the milk (b) stage. Four samples of dry corn were also collected: two (c and d) were of the same type, except that sample (d) had been stored for some time under unfavorable conditions. Two samples of corn meal were obtained in the market. Sample (a) was of a fine grind and the other (b) was a coarse meal.

Rice: *(Oryza sativa)*. Rice (arroz) is the preferred cereal of Cuba and is being grown here in increasing amounts. The bulk of the commercial supply comes from irrigated and mechanized plantations. Of the hundreds of varieties in the world, a half-dozen major varieties are planted in Cuba, mostly of the long-grain "Rexoro" type, [sample (a)], with some short-grain rice "Bolo" still grown in a few areas [sample (b)].

EARTH VEGETABLES

Arracacha: *(Arracacia xanthorrhiza)*. The arracacha (afió) is a native of the Andes and has spread northward to become a locally important starchy food in

TABLE 1

Composition of food samples from Cuba

Name of food sampled			Origin[1]	Constituents measured and yields per 100 gm.															
English	Scientific	Spanish		Moisture	Ether extract	Crude fiber	Nitrogen	Ash	Calcium	Phosphorus	Iron	Carotene	Thiamine	Riboflavin	Niacin	Ascorbic acid total	Tryptophan	Methionine	Lysine
				g.	g.	g.	g.	g.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
Cereals																			
Corn (a)	*Zea mays L.*	Maíz tierno	14	56.7	2.09	1.11	.81	.90	8.3	156.0	1.10	.23	.27	.10	1.72	9.9	27	106	158
Corn (b)	*Zea mays L.*	Maíz tierno	14	65.5	1.48	1.03	.75	.87	8.2	148.3	1.01	.10	.21	.10	1.98	11.6	35	84	151
Corn, dry (a)	*Zea mays L.*	Maíz seco	22	9.9	3.80	1.27	1.35	.96	6.0	163.0	1.63	.20	.35	.09	1.76	.....	46	147	270
Corn, dry (b)	*Zea mays L.*	Maíz seco	22	12.2	3.97	1.37	1.59	.95	7.1	184.0	2.08	.33	.35	.10	1.32	.....	33	163	236
Corn, dry (c)	*Zea mays L.*	Maíz seco	10	10.4	4.18	2.74	1.87	1.36	11.5	331.8	6.12	.34	.44	.12	1.24	.....	15	179	312
Corn, dry (d)	*Zea mays L.*	Maíz seco	10	3.4	3.61	1.68	1.51	1.23	20.5	243.0	4.57	.32	.51	.12	1.17	.....	33	105	239
Corn meal (a)	*Zea mays L.*	Maíz, harina de	23	9.1	4.34	.87	1.45	.74	13.3	136.4	6.93	.35	.18	.10	1.39	.....	56	191	218
Corn meal (b)	*Zea mays L.*	Maíz, harina de	23	6.0	3.64	.88	1.47	.44	9.4	86.3	1.81	.43	.09	.07	.61	.....	43	221	195
Rice, white (a)	*Oryza sativa L.*	Arroz	10	10.2	.51	.14	1.45	.43	5.5	97.2	1.73	....	.07	.04	1.62	.....	79	50	110
Rice, white (b)	*Oryza sativa L.*	Arroz	10	13.5	.41	.19	1.17	.46	7.8	111.4	1.10	....	.07	.03	1.74	.....	91	.....	260
Earth Vegetables																			
Arracacha	*Arracacia xanthorrhiza Bancr.*	Afió	23	75.8	.17	.35	.12	1.09	23.2	51.7	1.95	.25	.06	.03	5.30	21.3	5	2	26
Carrot	*Daucus carota L.*	Zanahoria	23	89.5	.28	.99	.16	1.02	38.0	47.4	1.24	3.70	.05	.05	.52	13.3	11	3	55
Malanga (a)	*Xanthosoma violaceum Schott.*	Malanga	24	66.4	.20	.73	.25	1.13	.....	57.3	1.43	.03	.12	.03	.....	9.6	.....	.....	.....
Malanga (b)	*Xanthosoma sagittifolium Schott.*	Malanga	24	74.3	.15	.75	.26	1.09	15.4	.....	.....	.04	.11	.03	.....	15.2	.....	.....	.....
Malanga (c)	*Xanthosoma sagittifolium Schott.*	Malanga	23	69.8	.29	.40	.26	.98	15.4	53.3	.64	.04	.12	.02	.44	10.7	22	15	64
Manioc (a)	*Manihot esculenta Crantz*	Yuca	20	58.0	.36	.81	.20	.65	24.6	40.6	.79	.03	.11	.02	1.08	50.0	.....	.....	.....
Manioc (b)	*Manihot esculenta Crantz*	Yuca	20	56.5	.06	.72	.17	.75	28.6	48.7	.67	.03	.10	.02	.72	31.8	.....	0	41
Manioc (c)	*Manihot esculenta Crantz*	Yuca	9	57.8	.27	.70	.13	.74	31.1	36.8	1.46	.04	.11	.05	1.23	30.9	9	2	43
Manioc (d)	*Manihot esculenta Crantz*	Yuca	2	56.6	.14	.73	.19	.52	22.5	38.9	.61	.03	.08	.02	.54	16.7	27	0	47
Manioc (e)	*Manihot esculenta Crantz*	Yuca	2	49.5	.18	1.32	.07	.64	22.5	43.2	1.01	.03	.06	.04	.75	48.6	23	1	35
Manioc (f)	*Manihot esculenta Crantz*	Yuca	13	58.3	.32	.69	.11	.83	29.7	37.4	.84	.85	.09	.03	.82	38.1	9	0	35
Potato	*Solanum tuberosum L.*	Papa	13	78.3	.05	.41	.30	.78	11.5	46.5	.....	.00	.07	.02	.....	57.2	.....	.....	.....
Radish	*Raphanus sativus L.*	Rábano	10	92.6	.03	.70	.21	.76	34.2	18.1	.67	.01	.01	.08	1.65	42.8	5	2	37
Sweet potato (a)	*Ipomoea batatas L.*	Boniato	1	35.8	.04	1.06	.25	.62	30.2	35.6	1.14	.00	.13	.04	1.56	43.7	22	29	79
Sweet potato (b)	*Ipomoea batatas L.*	Boniato	2	64.1	.08	1.22	.17	.63	14.2	30.2	1.07	.02	.15	.04	1.17	51.1	29	25	54
Sweet potato (c)	*Ipomoea batatas L.*	Boniato	6	60.4	.38	.85	.18	.50	17.9	43.2	.93	.01	.15	.04	1.35	33.1	10	9	33
Sweet potato (d)	*Ipomoea batatas L.*	Boniato	23	63.0	.28	.95	.39	.85	19.3	71.8	1.26	.01	.12	.04	.58	32.7	37	40	91
Taro	*Colocasia esculenta Schott.*	Malanga isleña	1	72.6	.12	.94	.24	1.15	89.5	116.1	1.03	.02	.11	.05	1.16	5.1	34	19	81
Yam (a)	*Dioscorea alata L.*	Name	24	75.6	.07	.45	.35	.71	12.3	40.5	1.09	.01	.12	.01	.....	9.0	.....	.....	.....
Yam (b)	*Dioscorea cayenensis Lam*	Name	1	54.2	.03	.35	.15	.43	7.7	26.7	1.04	.02	.08	.02	.32	13.2	14	31	59
Yam (c)	*Dioscorea cayenensis Lam*	Name	23	73.1	.19	.36	.42	.88	10.9	34.2	.96	.01	.10	.03	.32	7.4	47	23	136
Yam (d)	*Dioscorea alata L.*	Name	23	77.3	.03	.37	.26	.54	7.9	40.8	.45	.04	.08	.02	.33	6.7	27	10	68
Fruit Vegetables																			
Avocado (a)	*Persea americana Mill.*	Aguacate	3	78.6	11.13	2.51	.30	.87	9.5	47.8	1.21	.20	.07	.12	2.60	24.1	21	15	114
Avacoda (b)	*Persea americana Mill.*	Aguacate	3	86.0	4.69	2.13	.17	.60	15.3	27.8	.73	.14	.05	.08	.76	18.1	13	10	66
Avocado (c)	*Persea americana Mill.*	Aguacate	11	78.6	10.50	2.54	.24	.83	16.5	49.2	.94	.14	.07	.12	2.12	24.9	20	15	89
Avocado (d)	*Persea americana Mill.*	Aguacate	10	81.7	11.25	2.79	.22	.91	8.7	55.2	.85	.13	.07	.12	1.44	14.9	14	13	88
Avocado (e)	*Persea americana Mill.*	Aguacate	3	79.4	9.89	2.61	.24	.76	8.6	46.6	1.11	.18	.05	.14	1.90	21.6	20	16	92
Avocado (f)	*Persea americana Mill.*	Aguacate	10	77.7	11.20	.25	.17	.94	15.0	51.1	1.06	.25	.05	.11	1.97	18.4	9	11	67

[1] See Figure 1 (legend).

TABLE 1 (continued)

Composition of food samples from Cuba

Name of food sampled			Origin[1]	Constituents measured and yields per 100 gm.															
English	Scientific	Spanish		Moisture	Ether extract	Crude fiber	Nitrogen	Ash	Calcium	Phosphorus	Iron	Carotene	Thiamine	Riboflavin	Niacin	Ascorbic acid total	Tryptophan	Methionine	Lysine
				g.	*g.*	*g.*	*g.*	*g.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Avocado (g)	*Persea americana Mill.*	Aguacate	10	81.1	10.75	1.99	.26	.82	10.6	38.5	.79	.12	.12	.09	1.67	18.7	12	12	63
Avocado (h)	*Persea americana Mill.*	Aguacate	10	83.8	7.95	2.32	.24	.57	15.2	34.8	.91	.13	.04	.10	1.68	13.4	12	12	63
Avocado (i)	*Persea americana Mill.*	Aguacate	10	78.1	11.52	3.39	.17	1.09	15.8	49.8	.81	.10	.05	.07	1.75	28.1	15	11	67
Chayote	*Sechium edule Sw.*	Chayote	23	94.1	.27	.41	.11	.27	12.2	25.2	.35	.01	.01	.02	.44	15.9	9	1	44
Cucumber (a)	*Cucumis sativus L.*	Pepino	10	94.6	.07	.33	.15	.32	19.5	16.7	.55	.04	.03	.03	.28	18.0	6	5	38
Cucumber (b)	*Cucumis sativus L.*	Pepino	10	95.9	.19	.30	.11	.29	23.4	14.8	.37	.02	.02	.02	.20	15.8	6	5	30
Egg plant	*Solanum melongena L.*	Berenjena	23	93.8	.71	.69	.15	.41	14.8	21.2	.65	.01	.05	.04	.54	2.6	10	2	51
Okra (a)	*Hibiscus esculentus (L.) Moench*	Quimbombó	5	87.4	.15	1.00	.37	.75	93.3	55.4	.93	.32	.11	.15	1.04	35.3	20	20	114
Okra (b)	*Hibiscus esculentus (L.) Moench*	Quimbombó	5	86.8	.26	1.00	.42	.72	98.0	53.9	.92	.33	.04	.15	1.04	26.9	24	25	123
Pepper, green (a)	*Capsicum frutescens L.*	Ají	1	86.8	.28	1.03	.16	.51	9.9	31.0	.75	.30	.08	.06	1.10	170.0	10	9	63
Pepper, red (b)	*Capsicum frutescens L.*	Ají	1	89.7	.18	1.13	.17	.59	13.8	30.3	.85	1.68	.09	.18	1.49	160.0	.....	11	63
Plantain (a)	*Musa paradisiaca L.*	Plátano burro	16	74.4	.11	.40	.23	.63	12.2	32.5	.67	.06	.06	.04	.39	22.2	10	3	39
Plantain (b)	*Musa paradisiaca L.*	Plátano burro	16	71.9	.45	.37	.24	.63	11.3	31.4	.51	.11	.06	.05	.59	31.2	15	5	37
Plantain (c)	*Musa paradisiaca L.*	Plátano fongo	20	67.9	.05	.39	.20	.76	12.8	34.4	.72	.37	.08	.04	.49	54.2	.....	.....	.....
Plantain (d)	*Musa paradisiaca L.*	Plátano macho	20	60.8	.05	.52	.22	.76	.....	32.6	.13	2.00	.10	.05	.....	33.1	.....	.....	.....
Plantain (e)	*Musa paradisiaca L.*	Plátano macho	9	64.3	.15	.29	.18	.90	11.0	53.8	.98	.75	.07	.05	.55	23.2	7	7	55
Plantain (f)	*Musa paradisiaca L.*	Plátano macho	9	60.6	.78	.35	.14	.94	11.8	51.4	.93	.65	.05	.05	.42	24.5	12	7	60
Squash	*Cucurbita moschata Poir.*	Calabaza	3	86.1	.16	.55	.24	.72	9.4	33.0	1.05	1.10	.07	.05	1.40	22.0	14	8	44
Tomato, cherry (a)	*Lycopersicum esculentum var. cerasiforme Alef.*	Tomate	5	94.3	.31	.57	.20	.41	10.5	33.9	.57	.69	.10	.06	.64	40.3	8	10	56
Tomato (b)	*Lycopersicum esculentum Mill.*	Tomate	6	94.2	.22	.35	.17	.40	10.2	24.9	.90	.19	.08	.03	.45	19.4	5	6	44
Tomato (c)	*Lycopersicum esculentum Mill.*	Tomate	6	94.3	.51	.36	.19	.40	8.9	24.8	1.15	.52	.10	.05	.69	29.3	16	11	57
Wax gourd	*Benincasa hispida Cogn.*	Calabaza china	6	95.8	.07	.83	.08	.34	21.0	7.3	.48	.02	.00	.01	.29	26.8	3	4	11
Herbage Vegetables																			
Cabbage	*Brassica oleracea var. capitata L.*	Col	18	92.0	.16	.68	.16	.55	61.3	26.8	.15	1.02	.04	.03	.....	51.8	.....	.....	.....
Chard	*Beta vulgaris var. cicla L.*	Acelga	10	93.3	.27	.63	.38	1.13	176.2	36.4	2.35	2.03	.06	.06	1.04	71.9	26	0	120
Lettuce (a)	*Lactuca sativa L.*	Lechuga	10	95.8	.36	.54	.....	.78	55.0	21.8	2.39	1.26	.04	.09	.....	32.6	.....	.....	.....
Lettuce (b)	*Lactuca sativa L.*	Lechuga	23	94.5	.27	.71	.11	.95	89.4	26.3	3.79	.86	.06	.09	.....	21.0	.....	.....	.....
Lettuce (c)	*Lactuca sativa L.*	Lechuga	14	96.3	.36	.38	.18	.45	43.5	26.1	.99	.73	.07	.07	.31	16.5	11	4	66
Parsley	*Petroselinum crispum Mansf.*	Perejil	23	89.3	.96	.90	.40	1.43	206.0	50.5	3.18	5.65	.09	.25	.53	110.5	50	12	360
Watercress	*Nasturtium officinale R. Br.*	Berro	14	95.2	.41	.54	.29	.79	63.3	32.0	2.55	1.70	.05	.09	.36	37.1	22	7	101
Legumes and Oleaginous Seeds																			
Bean, black (a)	*Phaseolus vulgaris L.*	Frijol negro	10	13.0	.73	4.28	3.26	3.76	136.7	385.5	15.99	.00	.56	.19	2.22	.....	210	160	1510
Bean, black (b)	*Phaseolus vulgaris L.*	Frijol negro	21	19.0	1.40	3.59	3.15	3.80	123.0	201.0	14.27	.00	.81	.32	1.93	.....	320	104	1530
Bean, red (c)	*Phaseolus vulgaris L.*	Frijol colorado	23	13.3	1.37	3.60	3.21	3.58	84.8	205.6	6.52	.02	.48	.27	2.15	.....	290	108	1500
Bean, spotted red (d)	*Phaseolus vulgaris L.*	Frijol chino	10	11.0	.73	4.46	3.20	3.57	109.3	351.5	11.68	.01	.63	.20	2.04	.....	170	173	1410
Catjang, pea (e)	*Vigna cylindrica Skeels*	Frijol precioso	21	11.8	2.29	3.83	3.82	3.17	74.1	235.6	6.12	.01	1.08	.25	2.95	.....	314	165	1900
Catjang, pea (f)	*Vigna cylindrica Skeels*	Frijol precioso	17	12.4	1.13	4.07	3.35	3.45	64.7	498.6	6.75	.01	.92	.19	2.38	.....	270	268	778
Cowpea (g)	*Vigna sinensis Savi*	Frijol carita	10	14.2	1.73	5.02	2.85	3.18	51.9	408.8	6.69	.00	.63	.20	1.93	.....	280	263	1520
Green bean, asparagus (a)	*Vigna sesquipedalis Fruwirth*	Habichuela	3	89.5	.12	1.36	.54	.72	55.8	71.0	1.72	.60	.12	.14	.99	27.5	35	20	240
Green bean, asparagus (b)	*Vigna sesquipedalis Fruwirth*	Habichuela	10	88.5	.11	1.17	.52	.66	53.5	64.5	1.18	.47	.06	.10	1.14	24.3	31	20	158
Green bean, snap (c)	*Phaseolus vulgaris L.*	Habichuela	23	92.0	.37	.99	.29	.46	45.5	34.6	1.01	.48	.06	.10	.87	16.0	20	9	100
Green bean, sieva	*Phaseolus lunatus L.*	Frijol caballero	23	64.8	.41	2.33	1.67	1.65	42.7	136.3	3.20	.02	.24	.12	1.14	11.9	164	95	640

[1] See Figure 1 (legend).

TABLE 1 (concluded)

Composition of food samples from Cuba

Name of food sampled			Origin[1]	Constituents measured and yields per 100 gm.														
English	Scientific	Spanish		Moisture	Ether extract	Crude fiber	Nitrogen	Ash	Calcium	Phosphorus	Iron	Carotene	Thiamine	Riboflavin	Niacin	Ascorbic acid total	Tryptophan	Methionine	Lysine
				g.	g.	g.	g.	g.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
Peanut	*Arachis hypogaea L.*	Maní	3	10.0	41.00	5.18	4.75	2.33	67.2	444.0	5.19	.00	.89	.18	15.10	.5	302	249	1090
Sesame seed (a)	*Sesamum indicum L.*	Ajonjolí	17	4.5	51.90	4.56	3.81	4.25	1081.0	552.3	13.80	.01	1.15	.28	5.81	.....	260	720	350
Sesame seed (b)	*Sesamum indicum L.*	Ajonjolí	13	2.3	52.50	8.42	3.31	4.66	1167.0	559.0	21.80	.04	1.31	.26	5.83	.....	250	453	360
Fruits: Citric																			
Grapefruit	*Citrus paradisi Macfad.*	Toronja	8	90.5	.02	.77	.16	.41	9.2	19.8	.69	.01	.04	.01	.25	43.3	2	0	12
Lima	*Citrus Sp.*	Lima	6	90.5	.09	.16	.12	.27	13.5	15.9	.30	.01	.05	.02	.18	59.4	3	2	14
Mandarin orange (a)	*Citrus reticulata Blanco*	Mandarina	23	88.6	.29	.39	.11	.38	34.7	16.0	.49	.20	.11	.03	.28	29.0	7	5	27
Mandarin orange (b)	*Citrus reticulata Blanco*	Mandarina	8	90.6	.12	.36	.11	.32	27.8	13.5	.48	.04	.06	.02	.22	40.3	3	3	21
Mandarin orange (c)	*Citrus reticulata Blanco*	Mandarina	12	88.1	.09	.57	.11	.36	35.6	15.5	.88	.06	.05	.03	.26	36.2	3	3	25
Orange (a)	*Citrus sinensis (L) Osbeck*	Naranja	12	89.5	.03	.09	.13	.29	20.3	13.5	.78	.01	.04	.02	.22	45.2	1	1	15
Orange (b)	*Citrus sinensis (L) Osbeck*	Naranja	12	89.1	.04	.09	.09	.38	9.2	13.5	.59	.01	.07	.02	.22	65.8	2	2	19
Orange, navel (c)	*Citrus sinensis (L) Osbeck*	Naranja	8	90.9	.02	.04	.07	.34	8.9	18.6	.76	.01	.07	.02	.24	54.3	3	2	12
Orange, navel (d)	*Citrus sinensis (L) Osbeck*	Naranja	6	91.2	.05	.06	.08	.31	8.9	13.0	.43	.01	.07	.02	.24	48.8	1	1	13
Orange, valencia (e)	*Citrus sinensis (L) Osbeck*	Naranja	13	76.7	.19	.09	.12	.45	49.8	22.7	.59	.02	.10	.03	.....	61.2	.....	.....	.....
Fruits: Other																			
Banana, common (a)	*Musa paradisiaca var. sapientum Ktze.*	Guineo dátil	23	70.6	.22	.42	.26	.62	9.6	20.7	.98	.40	.02	.08	.49	11.9	30	21	83
Banana, common (b)	*Musa paradisiaca var. sapientum Ktze.*	Guineo morado	23	74.9	.34	.41	.20	.71	11.5	17.9	1.49	.42	.05	.05	.47	6.7	21	14	59
Banana, common (c)	*Musa paradisiaca var. sapientum Ktze.*	Plátano manzano	4	72.0	.08	.46	.18	.87	8.6	47.2	.77	.05	.05	.06	.73	20.9	15	7	54
Banana, common (d)	*Musa paradisiaca var. sapientum Ktze.*	Plátano puyá	15	75.0	.07	.55	.17	.71	9.5	22.0	1.07	.16	.04	.05	.66	7.1	13	6	41
Banana, dwarf (e)	*Musa nana Lour*	Plátano enano	4	78.1	.06	.33	.30	.64	13.8	29.9	.60	.27	.04	.06	.80	7.7	19	7	76
Coconut meat	*Cocos nucifera L.*	Coco, masa de	19	48.2	36.90	4.39	.63	.84	16.5	86.7	1.67	.00	.03	.03	1.01	6.9	.....	59	162
Coconut milk	*Cocos nucifera L.*	Coco, agua de	19	95.7	.24	.00	.01	.35	22.8	6.0	.29	.00	.00	.01	.05	1.6	0	0	3
Egg plant	*Pouteria campechiana Bachni*	Canistel	10	60.6	.13	.10	.27	.90	26.5	37.3	.92	.32	.17	.01	3.72	58.1	28	13	84
Guava (a)	*Psidium guajava L.*	Guayaba	7	80.3	.12	5.93	.19	.51	17.4	17.7	.99	.10	.05	.07	.65	484.0	5	8	25
Guava (b)	*Psidium guajava L.*	Guayaba	6	76.6	.12	5.39	.18	.68	17.6	28.2	1.16	.26	.05	.05	1.25	458.0	17	14	44
Mamey	*Mammea americana L.*	Mamey de Santo Domingo	10	86.4	.20	1.07	.07	.19	9.5	13.6	.50	.....	.03	.05	.32	12.5	5	6	35
Mango (a)	*Mangifera indica L.*	Manga	10	85.0	.51	.98	.02	.24	10.3	10.9	.46	1.41	.08	.10	.36	6.2	3	2	31
Mango (b)	*Mangifera indica L.*	Mango	10	82.0	.30	1.13	.08	.50	9.3	20.4	.79	.06	.05	.04	.58	17.4	5	4	42
Mango (c)	*Mangifera indica L.*	Mango	10	77.8	1.05	1.67	.09	.43	7.1	27.8	.51	2.52	.11	.08	.49	56.5	8	7	50
Mango (d)	*Mangifera indica L.*	Mango	10	85.5	.13	.65	.04	.35	5.2	10.7	.41	1.85	.04	.04	.36	94.1	4	2	26
Mango (e)	*Mangifera indica L.*	Mango	10	77.5	.65	.80	.04	.33	11.7	14.3	.44	1.63	.07	.04	.56	26.0	9	3	35
Melón	*Cucumis melo L.*	Melón de Castilla	8	95.0	.07	.23	.09	.30	8.3	8.1	.44	.95	.01	.03	.55	31.2	1	2	14
Papaya	*Carica papaya L.*	Fruta bomba	6	92.6	.05	.70	.04	.33	19.4	5.3	.78	.12	.03	.02	.25	43.9	5	1	16
Pineapple (a)	*Ananas comosus Merr.*	Piña	23	86.4	.21	.42	.09	.20	31.6	5.6	.31	.02	.08	.04	.17	90.5	7	2	13
Pineapple (b)	*Ananas comosus Merr.*	Piña	20	83.7	.10	.35	.04	.26	24.7	8.2	.....	.03	.08	.03	.....	75.8	.....	.....	.....
Soursop	*Annona muricata L.*	Guanábana	10	82.8	.97	.79	.16	.60	10.3	27.7	.64	.00	.10	.05	1.28	29.6	11	7	60
Sweetsop	*Annona squamosa L.*	Anón	10	72.0	.41	1.14	.28	.65	21.4	38.4	3.61	.01	.13	.16	.93	34.7	9	8	69
Miscellaneous																			
Sugar cane juice	*Saccharum hybrid POJ 2878*	Guarapo de caña	10	78.3	.05	.74	.06	.29	31.4	11.9	1.30	.03	.01	.02	.11	2.5	5	0	0
Sugar cane syrup	*Saccharum hybrid POJ 2878*	Melado de caña	10	42.5	.09	1.07	.05	1.04	126.9	9.3	1.19	.00	.02	.08	.44	.0	2	0	0

[1] See Figure 1 (legend).

several parts of the American tropics. In Cuba it is grown and consumed almost exclusively in the hilly region of the eastern part of the island. The one sample collected was bought at the market and showed its characteristic inside yellow color. It is commonly peeled, mashed, and eaten in the form of fritters and cakes.

Carrot: *(Daucus carota).* Carrots (zanahoria) are a relatively uncommon winter vegetable and are usually grown from U. S. seed of standard varieties. They are eaten, as in the U. S., raw in salads, or after boiling. The sample collected consisted of 12 units weighing 1200 g. bought at an eastern market.

Malanga: *(Xanthosoma violaceum* and *X. sagittifolium).* Malanga is the principal edible aroid of the New World and is equivalent to the taro of the Old World. The kind with purple stems and leaves called "malanga morada" (a) is often set off as a distinct species, *X. violaceum,* although it may be no more than a race of the much commoner "malanga blanca" *X. sagittifolium.* In the "blanca" race (b) and (c)—the lateral tubercles or "güagüies" are the part eaten, a division of the corm or "chopo" being used for replanting. Samples (a) and (b) were obtained from the fields and (c) from the market.

Manioc: *(Manihot esculenta).* Cassava or manioc (yuca), is the most widely grown root crop of tropical America. Numerous varieties are known which differ, among other things, in the amount of poisonous glucoside they contain. The slender lateral storage roots, which radiate around the base of the plant, are peeled and the poisonous cyanide driven off by the subsequent cooking procedures. The roots are used in as many ways as are potatoes. The bitter variety is a prime source of tapioca starch which is commercially extracted in small factories. The historic cassava bread, also called by the original Indian name "casabe," is widely used in eastern Cuba. Six samples of cassava were collected. Samples (a) and (b) belong to a variety called "negroncita," the (b) corresponding to two-year-old roots and the (a) to younger roots. Sample (c) called "negrita," consisted of 5-months-old roots and (d) of the "señorita" type somewhat older, possibly 7 months. Sample (e) called "pana" (meaning compact) was around 11 months old. Sample (f) consisted of 6 units weighing 2900 g. and consisting of young roots the precise age of which was not known. All samples were collected from the fields in different parts of the island.

Potato: *(Solanum tuberosum).* Potatoes (papa) are grown in Cuba from disease-free seed pieces of standard varieties imported annually from Canada and the United States. The one sample was a redskinned variety growing on fertilized and irrigated land, the common practice with this seasonal and high-value crop.

Radish: *(Raphanus sativus).* Radishes (rábano) are commonly grown in winter vegetable gardens, usually from U. S. seed. This sample was of a white-fleshed and white-skinned variety grown by Chinese market gardeners and occasionally sold erroneously as "nabo."

Sweet potato: *(Ipomoea batatas).* Four samples of sweet potato (boniato) were collected. The mature tubers were peeled and sliced for subsampling. Samples (a), (b), and (c) were collected in the fields, while (d) was bought at the market. Although new fields are always planted with vine cuttings, countless "varieties" of the plant are found because it seeds freely. Varietal common names refer actually to seedling races, some of which are fairly well marked; however, different names are applied to this plant in different parts of the island. White-fleshed forms are preferred. They are prepared for eating in the ways familiar in the United States.

Taro: *(Colocasia esculenta).* The Old World taro (malanga) isleña is less commonly grown in Cuba than the native "malanga," but is preferred by the many Canary Islanders and their descendants who are a small but important element of the agricultural population. The rhizome or corm, called variously the "chopo" or "ñame" (which should not be confused with the plant called "ñame"), is the part eaten, while the lateral tubercles, called "bolas" or "güagüies," are used for replanting the crop. The starchy roots are cooked as a vegetable in various ways, or used in soups and stews.

Yam: *(Dioscorea alata* and *D. cayenensis).* These 2 species of the true yam (ñame) are botanically quite distinct from the sweet potatoes called yams in southern United States and were introduced into Cuban agriculture from Africa during early slave days. Native species of *Dioscorea* exist but are seldom used for food. The various

kinds of yams in cultivation, mostly in the easternmost part of the island, are grown on stakes in regularly spaced hills and the abundant vines die back annually as the tubers mature. Four yams were collected, samples (a), (c), and (d) were white, and sample (b) was yellow. Samples (c) and (d) were obtained from the market in the eastern part of the island. The tubers are pared and usually cooked by boiling, the end portion being retained for replanting.

VEGETABLES

Fruit

Avocado: *(Persea americana)*. The avocado (aguacate) is used chiefly as a vegetable in Cuba, either sliced, or in salad. Almost all are from seedling trees, although there is a growing tendency to establish orchards of superior grafted varieties. All of the 9 samples collected are of the West Indian race.

Chayote: (Sechium edule). Native of tropical America, the "chayote" is a common dooryard vine throughout the world tropics. Its smooth or soft-spiny, pear-shaped fruits are either green or white, and are used in a variety of ways. Although the roots, leaves and even the flowers are eaten in Central America, no evidence of such practices has been found in Cuba. The sample collected was obtained from the market.

Cucumber: *(Cucumis sativus)*. The cucumber (pepino) is used extensively as a raw salad vegetable in Cuba and is grown both for domestic use and for export to the U. S. Sample (a) consisted of 9 units weighing 870 g. and was collected in the fields, while (b) was obtained from the market.

Eggplant: *(Solanum melongena)*. The eggplant (berengena) is grown in large quantities for export, as well as for domestic consumption. Most seed is of U. S. origin and the fruit is prepared in familiar ways. Purple and green-skinned varieties are seen. The one sample collected was obtained in the market.

Okra: *(Hibiscus esculentus)*. Okra (quimbombó) has an established, though relatively minor, place in the agriculture of the country and was introduced from Africa during the slave period. It is used in soups, as a special stew, and as a boiled green vegetable. Two samples were collected, one of the long type (a), and another one of the short type (b).

Peppers: *(Capsicum frutescens)*. Small conical-fruited capsicum peppers (ají) are commonly grown for use in seasoning and in the preparation of sauces. The 2 samples collected were from the same plants, one sample (a) of green and the other (b) of fully-ripe red fruits. Peppers are native to the New World and the classification of varieties has not yet been worked out.

Plantains: *(Musa paradisiaca)*. The name "plátano" is applied to both plantains and bananas indiscriminately in Cuba, but is used here to refer to the plantains alone, bananas being discussed under that name in the section on fruits. Plantains are distinguished from bananas chiefly by the fact that they must be cooked to be edible. The two most important kinds in Cuba are "burro," samples (a) and (b), and "macho" (d), (e), (f) and also (c) under the name "fongo." Samples (a) and (b) were from the same stalk but the first was green and the second was ripe. Similarly, sample (3) was the unripe, and sample (f) the ripe fruit of another plant. There are many ways of preparing these for the table. They may be baked, boiled or fried in shallow or deep fat, the method of preparation depending to some extent on the ripeness of the fruit.

Squash: *(Cucurbita moschata)*. The classification of the gourd fruits is continually undergoing changes and the present designation reflects the current thinking of specialists in the field. The sample collected was a common squash (calabaza) grown throughout Cuba, of the group called the soft-leaved winter squashes. It is a large, round, hard-shelled, green-skinned fruit, with yellow flesh.

Tomato: *(Lycopersicum esculentum)*. Sample (a) consisted of a cherry tomato, "tomato placero," a small fruited perennial, semi-wild plant widely grown in Cuba for its fruits. These are used for making sauces when fully ripe, and less commonly are peeled and used for salad. During the dry season, the large-fruited annual salad tomatoes are grown for domestic consumption and for export. These are almost always of standard U. S. varieties grown from imported seed. They are usually eaten sliced in salad, while still quite green, as in sample (b) or, much more rarely, ripe as in (c).

Wax gourd: *(Benincasa hispida).* Chinese preserving melon or wax gourd (calabaza china) is an unusual food material, grown principally by Chinese gardeners and eaten in the form of the candied flesh.

VEGETABLE

Herbage

Cabbage: *(Brassica oleracea* var. *capitata).* Cabbage (col) is a common winter vegetable grown throughout the island from seed which is usually of U. S. origin. This collection was of the common, large-headed, green kind locally called "col tambor." It is usually used cooked as a vegetable or in soup, but occasionally is eaten raw in salad.

Chard, Swiss: *(Beta vulgaris* var. *cicla).* The large leaves and thick leaf stalks of swiss chard (acelga), a form of beet, are commonly used as cooked vegetable or in soups. It is of particular interest because it is one of the few green vegetables that are available the year round. The one sample was of the preferred pale, smooth-leaved variety.

Lettuce: *(Lactuca sativa).* Lettuce (lechuga) is usually grown in the dry winter season from U. S. seed and has a growing popularity as a salad green. Three kinds were sampled: plain-leaf (a), curly-leaf (b), and head lettuce (c). The last is seldom grown successfully because of a tendency to bolt early.

Parsley: *(Petroselinum crispum).* Parsley (perejil) is a minor truck crop, grown chiefly for use in seasoning, and as a decoration of food. Although the curly-leaf kind is sometimes seen, the flat-leaf is commoner. The flat-leaf sample was obtained from the market.

Watercress: *(Nasturtium officinale).* Watercress (berro), a favorite salad green throughout the island, is grown under heavy natural or artificial irrigation and is marketed throughout the year. It is native to Europe but is now widely naturalized in streams and marshes throughout the world. The sample collected consisted of whole young plants, the roots being removed in the preparation.

LEGUMES AND OILSEEDS

Beans (Dry)

Bean, common: *(Phaseolus vulgaris).* The common bean (frijol común) is native to the New World and was growing in Cuba when Columbus first landed. It occurs in a diversity of forms distinguished commonly by the color and the marking of the seed coat. Relatively few kinds are grown in Cuba in comparison with other countries such as Mexico. Four samples of common bean were collected: (d) spotted red bean, called *"frijol chino"* or *"chileno,"* (e) red kidney bean, called *"frijol colorado,"* both obtained from the market, (a) the favorite black bean called *"frijol negro,"* collected from the current crop and (b) collected from the market. The same beans are occasionally harvested earlier as string beans under the name "habichuela catalana" (see below). Much remains to be done to improve the yield and other characteristics of this important crop in Cuba.

Catjang pea: *(Vigna cylindrica).* The catjang or Hindu cowpea (frijol precioso), is a rare crop in Cuba, although much appreciated in some localities for its edible seeds, which are used in the same manner as are the common beans. The plant is like the common cowpea and may even be a race of it, but the seeds lack the black "eye." Two samples were obtained: sample (e) consisted of dry seeds of medium to poor quality, which were being sold, quite erroneously, as soybeans; sample (f) was of good quality.

Cowpea: *(Vigna synesis).* The cowpea or blackeyed pea (frijol carita), native to Asia, is widely marketed in Cuba although it is not much appreciated as a dry bean except when prepared in the form of small cakes called "bollos." The cowpea is also planted as a forage plant and as a green manure. This collection (g) was obtained from the market.

Green Beans

Bean, asparagus: *(Vigna sesquipedalis).* The yard-long or asparagus bean (habichuela china) is one of the cowpea group of beans, and is distinguished from the others

by the greatly elongated and flaccid mature pod. The long, slender, round immature pods are the standard string bean of the country, and are available the year round. They are cooked for use as a vegetable or in salads. Two samples were collected in the fields, sample (a) consisting of 20 units weighing 643 g. and (b) of 150 units weighing 700 g.

Sieva bean: *(Phaseolus lunatus).* The sieva bean (frijol caballero) is a close relative of the lima bean *(Ph. limensis)* and is often confused with it. It is more common in domestic markets. The beans are marketed both green and dry, and appear in a variety of colors: pink, green, white, and mottled. The one sample analyzed was of the white type.

Bean, snap: *(Phaseolus vulgaris).* String or snap beans (habichuela catalana), the immature and entire pods of the common bean, are seldom eaten in Cuba. The asparagus bean is preferred, because it is more resistant to local diseases and can be grown in all seasons. Sample (c) consisted of beans of good quality obtained from the market.

Peanut: *(Arachis hypogaea).* The peanut (maní) is native to South America, and was introduced into Cuba during slave days from Africa, where it had been carried by the early Portuguese traders. There has been a great extension of planting of this crop as an oil seed in recent years. Despite the varying fortunes of the industry, it continues to be important, both as a roasted seed and as a source of edible oil. The sample collected consisted of the red-skinned type.

Sesame: *(Sesamum indicum).* Sesame (ajonjolí) is grown in Cuba on a rather local and limited scale, although it is generally known. The plant is a low, erect annual, easy of cultivation, and of remarkable adaptability. It is native to southern Asia and is extensively grown as the source of sesame oil, not only in China and India, but also in Africa and in various parts of Latin America. It was probably first introduced in Cuba from Africa during the slave days. In Cuba, the whole seeds are used in a candy, and in a tonic beverage called "horchata," reputed to be of particular benefit to lactating women. It is of no importance yet as an oil seed. Two samples were collected: from the market (a) and from the field (b).

FRUITS

Citrus

Grapefruit: *(Citrus paradisi).* Several grafted varieties of grapefruit (toronja) are grown in plantations for export, while a host of seedling types are found throughout the island. The juice is used more as a tonic than as a refreshment, and the halved fruit is rarely eaten in the U. S. manner. The name "toronja" is sometimes applied to the pummelo (Citrus grandis), the thick-skinned and presumably parental form of the grapefruit which is much less common in Cuba; presumed intermediates are occasionally seen. The sample of grapefruit consisted of 8 units weighing 5,000 g. from which the juice and part of the pulp were removed and slurried.

Lima: *(Citrus* Sp.) The Cuban "lima" is probably a sweet lime hybrid, but the classification of citrus fruits is still so poorly understood that it is not possible yet to identify it with any greater precision. The fruit is thin-skinned, is like an orange in appearance, and has a pale and rather insipid juice which is used as a beverage. It is not common and is becoming less so. The sample consisted of 12 fruits weighing 2,050 g.

Orange: *(Citrus sinensis).* Oranges (naranja) have long been the most popular citrus fruit in Cuba and extensive groves exist in many parts of the island. The "Navel" (c) and (d) and "Valencia" (e) varieties are the most common grafted kinds grown for market, but dozens of other varieties and seedling strains (a) and (b) are also found. Sample (e) was prepared by peeling all of the white rind which covers the orange, and slurrying the separated segments. The other samples were cut through the middle, the seeds and center removed, and the juice and part of the pulp expressed with an electric juicer.

Orange mandarin: *(Citrus reticulata).* Several varieties of the mandarin orange (mandarina) group are planted in Cuba, particularly the tangerines and the hybrid king oranges. All are characterized by a loose reddish-orange peel and easily-separated segments. Sample (a) consisted of 12 units weighing 1,435 g., were peeled by hand,

the seeds removed, and the segments slurried. Sample (b) was of the variety called "Naranja de San José," although it is a true mandarin. Sample (c) was bought at the market and prepared in the same way as (a) and (b).

FRUITS

Banana: *(Musa paradisiaca* var. *sapientum).* The common banana (plátano) is widely grown in Cuba for the fresh fruit. This and the dwarf banana are often referred to as "guineos" or "plátanos guineos" in some parts of Cuba, to distinguish them from the plantains. The best varieties, such as "Johnson," which is the "Gros Michel" of the Central American commercial plantations, and the "manzano," have suffered heavily from various diseases and insect pests. Both sample (a) of the variety "guineo datil," and sample (b), the variety "guineo morado," were obtained at the market; whereas sample (c) "plátano manzano" widely used in the island, and sample (d) "plátano puyá" were obtained from plantations.

Banana, dwarf: *(Musa nana).* The dwarf banana (plátano enano), including the variety "congo," is of increasing importance in Cuba because of its resistance to the Panama disease which has practically eliminated the standard commercial variety of the common banana. The name "dwarf" refers to the short pseudostem and not to the actual fruits, which are of good size [Sample (e)].

Coconut: *(Cocos nucifera).* The coconut (coco) is losing ground in Cuba as the result of the attacks of insects and diseases, but it is still consumed in large quantities. The meat, "masa de coco," is taken from the mature fruit, while the milk, "agua de coco," is drawn from the immature nuts. Several varieties are planted, principally the green-fruited "blanco," and the yellow-fruited "indio"; the latter is increasing in popularity because of its reputed disease resistance.

Egg Plant: *(Pouteria campechiana).* The egg plant (canistel) is cultivated throughout most of Cuba and has become naturalized. Although the perfectly ripe fruit is sweet and aromatic, it has not won very wide acceptance. It is suitable for use in a number of ways, both fresh and cooked. The sample collected had the characteristic yellow pulp, resembling egg yolk in color and texture, which gives the fruit its common name.

Guava: *(Psidium guajava).* The guava (guayaba) is an ancient cultivated plant in the New World, including Cuba, and has spread widely. It is found in several races, the most appreciated of which is called "cotorrera," with a highly aromatic rosy flesh and a thick yellow rind. The "blanca" race is paler and has less flavor. Most of the fruit is used in the production of jellies, marmalades, and pastes in numerous small factories throughout Cuba. Certain kinds are popular as fresh fruits in the rural areas. Sample (a), corresponded to the "cotorrera" type, and sample (b), which was the "blanca" type, both were obtained from the field.

Mamey: *(Mammea americana).* The mamey or mammee apple (mamey de Santo Domingo) is native to the West Indies and northern South America and is rather variable in quality, since practically all trees are seedlings. In Cuba it is used rarely as a fresh fruit, its principal use being for preserves.

Mango: *(Mangifera indica).* The mango (mango, manga), a native of India, has become so thoroughly naturalized in Cuba as to appear native. While many superior kinds are propagated by grafting, most of the trees outside of the commercial plantations are seedlings and belong to several races. A major distinction is made between "mango," with elongated, somewhat kidney-shaped, fruit and long leaves, and "mangas," which have rounded or heart-shaped fruit and shorter leaves. Many superb seedling trees exist which should be perpetuated by grafting, but little has been done. The fruit is usually eaten fresh, but is also made into a preserve. Sample (a) was "manga blanca," (b) a "mango jamaiquino," (c) a "mango jobo," (d) a "mango macho" and (e) a "mango piña." They were all taken in the field.

Melon: *(Cucumis Melo).* The melón (melon de Castilla) is an ancient cultivated plant in the temperate regions of the Old World. It is occasionally planted in Cuba as a winter fruit, although not as commonly as the watermelon. It exists in several varieties, chiefly the so-called cantaloupe or muskmelon, and the honeydew. The seed is usually of U. S. or Spanish origin.

Papaya: *(Carica papaya).* The papaya (fruta bomba) is probably of Middle American origin, and is widely cultivated in the island, where it frequently escapes from cultivation. The recent spread of a serious virus disease has limited the production of fruit considerably, but it is still common in the market and in domestic use. It is extremely variable in form and quality, all plants being seedlings. The sample consisted of 2 fruits which weighed 13 pounds.

Pineapple: *(Ananas comosus).* Pineapples (piña) have been grown in Cuba perhaps from pre-Columbian times and now are extensively grown wherever appropriate soils exist. They have high-value as export and considerable quantities are used domestically. Two principal varieties are grown. The "morado" is a red-fruited kind which ships well, and the "blanca" is sweeter and more flavorful but is too thin-skinned for export. It is used as fresh fruit, as juice, and in several forms of preserves. Both samples were of the "blanca" variety; (a) was obtained from the fields and (b) from a market in the eastern part of the Island.

Soursop: *(Annona muricata).* The soursop (guanábana) is a native of the West Indies with large, soft-spined fruits containing a white pulp with a pleasantly aromatic acid flavor, and from which a beverage and ice cream are made.

Sweetsop: *(Annona squamosa).* The sweetsop or sugar-apple (anon) is a favorite fresh fruit in the island wherever it has survived the devastating attacks of an insect borer. It is native to the West Indies and South America and has been widely planted. The one sample collected was from the field.

MISCELLANEOUS

Sugar cane juice and syrup: *(Saccharum hybrid).* The mill juice (guarapo de caña) from the first crusher rolls and the concentrated uncrystallized syrup (melado de caña) of the sugar cane were collected at a commercial sugar mill during regular processing of the variety POJ 2878. This is the standard cane variety of Cuba, accounting for more than 60% of the crop.

DISCUSSION OF RESULTS

More than one sample of many of the foods were collected and analyzed. It is of interest to compare the values of these different samples, and to point out those with outstanding nutrient values. Since samples of the same food varied in moisture content, the comparison has been made on a dry weight basis.

CEREALS

Corn, dry: The iron content of sample (c) was definitely higher than that of the other 3 samples, but the vitamin values were quite similar. It is to be noted that the vitamin values of dry corn were definitely lower (on a dry weight basis) than those of green corn.

Corn, green: The 2 samples of green corn were similar in composition. The higher carotene content of sample (a) may not be significant.

Corn, meal: The ash, fat, iron, calcium, phosphorus, thiamine, and niacin content of sample (a) was higher than sample (b).

Rice: Even though the 2 rices analyzed were different, the values reported were very similar. The B-complex vitamin contents were low, as expected in a refined cereal. The amino acid values were also low, but the lysine content of sample (b) was twice as high as sample (a), 260 and 110 mg. per 100 g., respectively.

EARTH VEGETABLES

Arracacha: The one sample of arracacha had a weak yellow color. It contained a very little carotene (.25 mg. %); and its niacin value was outstandingly high (5.30 mg. %).

Carrot: The carrot sample showed the high value in carotene (3.70 mg.) typical of carrots analyzed elsewhere.

Malanga: The composition of three samples was quite similar, while that of the fourth sample (taro) was different in several respects.

Manioc: Six samples of cassava were analyzed. The mineral values were the same, except that the iron content of sample (c) was unusually high. The fiber content of sample (e) was high also. The cassava is a food unusually poor in protein content.

Potato: Only one sample of potato was analyzed and it was especially rich in ascorbic acid (57.2 mg.). This may be of considerable nutritional importance especially because large amounts of potato can be eaten in the daily diet.

Sweet potatoes: The 4 samples of sweet potatoes showed approximately the same composition, except that sample (d) was low in niacin and sample (e) was low in tryptophan, methionine, and lysine.

Yam: Four samples of yam were very similar. Although the yellow yam, sample (b), was yellow in color the carotene value was extremely low (.02 mg.), indicating that the color was not due to carotene.

VEGETABLES

Fruit

Avocado: Nine different types of avocados were studied. Their composition did not vary greatly. The only exceptions were in ether extract and niacin values.

Cucumber: Both cucumber samples showed similar composition, and none of the nutrients were present in important amounts.

Okra: Small differences were noted between the two samples of okra analyzed, the only important variation was found in thiamine (.11 and .04 mg. %).

Pepper: The two samples of pepper differed greatly in carotene (.30 and 1.68 mg. %) and in riboflavin (.06 and .18 mg. %) content. In both cases the red pepper was the higher. No important differences in the other nutrients were found. Both samples were high (170 and 160 mg. %) in ascorbic acid.

Plantain: The six samples of plantain varied in ether-extract, and especially in carotene content. The green or unripe "plátano macho" had a significantly higher carotene value. Plantains were poor in mineral and protein content.

VEGETABLES

Herbage

Chard: The one sample of chard studied showed high content of calcium (176.2 mg. %), iron (2.35 mg. %), carotene (2.03 mg. %), and moderately high content of ascorbic acid (71.9 mg. %). It was very low in protein content, and the methionine value was nearly zero.

Lettuce: Of the 3 types of lettuce analyzed, sample (b) had highest values in calcium (89.4 versus 55.0 and 43.5 mg.%) and iron (3.79 versus 2.39 and .99 mg. %). The carotene values for all 3 samples were appreciable (1.26, .86, .73 mg. %).

Parsley: The leafy parsley was rich in calcium (206.0 mg. %), iron (3.18 mg. %), carotene (5.65 mg. %) and ascorbic acid (110.5 mg. %). Its lysine value was unusually high (360 mg. %).

Watercress: The watercress sample showed outstanding values for iron (2.55 mg. %) and carotene (1.70 mg. %).

LEGUMES AND OLEAGINOUS SEEDS

Beans: The bean samples showed considerable variation in the nitrogen content. The iron values were outstandingly high. The thiamine content was high, with only a small variation (.56, .81, .48, .63, 1.08, .92, and .63 mg. %). The highest values were found in samples of Hindu cowpea (e and f). The niacin content of the Hindu cowpea was also outstanding (2.95 and 2.38 versus 2.22, 1.93, 2.15, 2.04, and 1.93 mg. %). The lysine values of sample (e) reached 1,900 mg. %, and the tryptophan content of all samples, except "frijol chino," were above 200 mg. %. The methionine value was relatively low and fluctuated between 268 and 104 mg. %. In general, the Hindu cowpea and the black beans, even though belonging to different genera were outstanding among the bean samples.

Green bean: Of the samples of string bean and yard-long bean analyzed the nitrogen values for samples (a) and (b) were higher than (c) (.54, .52 *vs.* .29 mg. %). The mineral content of these samples was only fair: calcium (55.8, 53.5, 45.5 mg. %), phosphorus (71.0, 64.5, 34.6 mg. %), and iron (1.72, 1.18, 1.01 mg. %). The carotene values were not high, and showed some variation (.60, .47, and .48 mg. %).

Sieva bean: The one sample of sieva bean (frijol caballero) analyzed showed modest amounts of iron (3.20 mg. %), thiamine (.24 mg. %), and lysine (640 mg. %).

Peanut: Even though only one sample of peanut was studied it is important to point out that it contains many valuable nutrients: fat (41.0%), nitrogen (4.75%), phosphorus (444 mg. %), iron (5.19 mg. %), thiamine (.89 mg. %), riboflavin (.18 mg. %), and especially niacin (15.1 mg. %). The peanut was the richest niacin source analyzed. The relatively high tryptophan (302 mg. %), methionine (249 mg. %), and lysine (1,090 mg. %) contents indicate that it contains a high quality protein.

FRUITS

Citrus

Mandarin: Of the 3 mandarin oranges analyzed samples (b) and (c) showed moderately high content of ascorbic acid (40.3, 36.2 mg. %) and wide variation in carotene content (.20 mg. *vs.* .04 and .06 mg. %).

Orange: Five samples of orange (naranjas) were analyzed. The ether extract content of the "Valencia" (e) was higher than the rest (.19 *vs.* .03, .04, .02, and .05 mg. %) and it was also higher in calcium content (49.8 *vs.* 20.3, 9.2, 8.9, and 8.9 mg. %). All samples contained significant amounts of ascorbic acid (45.2, 65.8, 54.2, 48.8, and 61.2 mg. %).

FRUITS

Bananas: The 5 bananas analyzed showed no outstandingly high values. Some samples contained modest amounts of carotene (.40, .42, .05, .16, .27

mg. %). The lowest value was obtained on sample (c) which is widely consumed in the western part of the island. The ascorbic acid content varied considerably (11.9, 6.7, 20.9, 7.1, and 7.7 mg. %).

Coconut: The coconut meat had a high content of fat (36.9%). The milk was higher in calcium than the meat (32.8 *vs.* 16.5 mg. %) and the iron higher in the meat than in the milk (1.67 *vs.* .29 mg. %).

Eggplant: The one sample of eggplant analyzed had as much ascorbic acid as an orange (58.1 mg. %) and moderately high amounts of niacin (3.72 mg. %).

Guava: The 2 samples of guava analyzed showed the highest fiber content (5.93 and 5.39 mg. %) of all the samples analyzed. The ascorbic acid content was exceptionally high (484 and 458 mg. %), evidence that it can be a good dietary source of this vitamin.

Mango: The 5 samples of mangoes analyzed showed variations in carotene (1.41, .06, 2.52, 1.85, and 1.63 mg. %); the "mango jobo" sample (c) being the highest. Also, the ascorbic acid values fluctuated (6.2, 17.4, 56.6, 94.1, and 26.0 mg. %), with sample (d) giving the highest value.

Pineapple: Two samples of pineapple were studied and they proved to be of importance only as a source of ascorbic acid (90.5 and 75.8 mg. %).

Sweetsop: The sweetsop sample had a significant quantity of iron (3.61 mg. %) and amounts of thiamine (0.13 mg. %) and riboflavin (0.16 mg. %) which are unusually high for fruits.

SUMMARY

A total of 115 samples of foods of vegetable origin was collected in different parts of Cuba during the period February 1953 through December 1953. The majority of the samples were obtained directly from the lands where they were cultivated, and a few were taken from the market place.

Botanical descriptions of the different samples and their Spanish, English, and scientific names are given. Descriptions of the foods and the dishes in which they are consumed in the areas where they were collected are also presented.

These samples were analyzed for moisture, ether extract, crude fiber, nitrogen, ash, calcium, phosphorus, iron, carotene, thiamine, riboflavin, niacin, ascorbic acid, tryptophan, methionine, and lysine content.

The results of the analysis of these samples of cereals, earth vegetables, fruit vegetables, herbage vegetables, legumes, oil seeds, and fruits are presented and evaluated.

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DESCRIPTIVE TERMS AND POINTS SYSTEMS FOR RATING FOOD QUALITIES

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Ehrenberg *(4)* has raised some important points in his criticisms of Plank's "Rational Method of Grading Food Quality" *(10)*, but perhaps his most interesting point occurs when he queries Plank's statement—"it is reasonable to assume that the difference in quality between good and fair is equal to that between fair and poor." This raises the whole question of whether scales based on 'equal sensory intervals' can be regarded as "linear," in the sense for which this term is applied to physical measurements and, further, whether scales based on descriptive terms can be regarded as having equal intervals even in terms of "equal sensory intervals."

There has already been much discussion on this question by psychologists and by those concerned with subjective assessments of food quality. Thus, Lickert *(9)* maintains that as good results can be obtained by treating ratings as if they were linearly related, as by scoring them in sigma units.

Bate-Smith *(1)* and Stevens *(16)* do not consider that ratings are a form of measurement for which anything but very simple statistics should be used, but Burke *(3)* and Ehrenberg and Shewan *(5)* consider that it does not matter if ratings do lack certain measurement properties, such as additivity or equality, and that advanced statistics may justifiably be used for them.

Quite apart from this statistical question, however, most writers have definite opinions as to whether or not equally spaced grade numbers or arbitrarily ordered descriptive terms imply equal intervals of quality. Thus, Hicks *(7)* maintains that it is difficult to achieve linearity with a rating scale consisting entirely of descriptive terms, but Plank *(10)* considers it "reasonable to assume that the difference in quality between good and fair is equal to that between fair and poor." It is this latter statement which Ehrenberg criticizes, maintaining that, as it stands, it can neither be verified nor disproved.

In fact, however, both Lickert and the writer have attempted to investigate this question of linearity by experiment, although Lickert's decision to consider certain descriptive terms as forming a linear scale seems to have been based more upon convenience than upon any true interpretation of the meaning of these descriptive terms.

THE RELATIONSHIPS BETWEEN DESCRIPTIVE TERMS

The writer has attempted to find out what the relationship between descriptive terms in a rating scale is held to be by the judges themselves, by the use of a suitably designed questionnaire *(14)*. Thus, with terms such as *fairly good, very good, extremely good,* etc., it was found that the interpretation varied a great deal between judges; that linear relations between the terms are not found even when the interpretations of a large number of judges are averaged; and that the interpretations of certain terms will depend

to a large extent upon the order in which they are presented in the rating scale. Thus *rather good* may readily be interpreted as either better or worse than *good*, depending upon the way it is presented.

In the questionnaire, the subjects were asked to mark in each descriptive term at what they considered appropriate positions on a straight line extending from a mark termed *extremely good* to a mark termed *extremely bad*, "as if equal distances along the line represented equal increases, or decreases, in quality." Now Ehrenberg criticizes Plank by saying that he seems to assume that quality has already been quantitatively measured and, further, says that Plank assumes that it is already known what an "equal difference in quality" means.

This problem of definition is of course avoided in the writer's questionnaire, but becomes more relevant if it is intended to apply these results to ratings for real qualities. This proved to be possible and, in fact, it seems that judges are quite able to think and judge in terms of 'equal quality intervals.' Thus, the term 'equal quality intervals' has meaning for the judges, though it may not be possible to define what they mean by it, if neither suitable words nor an instrumental method of measurement can be found. In any case, when only subjective assessments are to be used, and it can be shown that judges agree and can make consistent assessments, it is not essential to ask what they "mean" by "equal quality intervals."

When subjective assessments are to be related to measurements with physical instruments, however, as in research carried out at the National Institute for Research in Dairying, it may be important to know the relation between subjectively "equal intervals" and instrumentally equal intervals, since such instrumental measurements are likely to have the properties of additivity and equality. It will also be important to know the relation between the descriptive terms, so as to know whether the scale is "linear" in terms of the subjectively "equal" quality intervals.

The writer has obtained some evidence on this, and it is worth noting that by use of the questionnaire described it was possible to draw a much smoother curve relating subjective assessments for the spreadability of butter to a corresponding instrumental measurement. When the non-linearity of the descriptive terms was accounted for, it seemed that the subjective "equal" quality intervals and the instrumental "equal" quality intervals bore a simple relation to each other *(11, 14)*.

It is perhaps worth noting that if scores are given for each descriptive term, in the rating scale which is used by the judges, some judges may rate primarily in terms of the scores and thus use a linear rating scale, while others may rate in terms of the descriptions and will therefore probably not be using a linear rating scale.

Quite apart from this question of linearity, there are some further points raised by Plank which are worth considering. Thus Plank discusses two alternative sets of descriptive terms, in one of which the term *very good* corresponds to grade 8 and in the other to grade 9. A [illegible] term such as *rather good* can change in meaning according to it[illegible] qualities which might normally be described as *extremely good* wi[illegible] as *very good* if a term *extremely good* is not given with the rating [illegible] such a term as *very good* can *change* in meaning, however, or whether it may [illegible] have its meaning *extended* in one or perhaps both directions, by the omitting of other descriptive terms, is an interesting question.

Plank also states that a scale should be restricted to five or ten grades "according to the sense thresholds of the tasters." In practice, however, it is often possible for judges to discriminate more than five or ten differences in the total range of samples, but it is because a series of typical qualities, and the grading numbers or descriptions allotted to them, have to be remembered, that it is inadvisable to use more than this number of grades *(13)*. With a small number of samples, assessing relative differences appears to be a psychologically "simpler" task than making quality ratings and it is as well that the two procedures should not be confused. There is even some evidence to suggest that the judges who are most skilled in the detection of relative differences may not be the most consistent when making quality ratings *(16)*.

OTHER ASPECTS

That there can be other ways of using scoring systems is shown by Ehrenberg's discussion of Plank's article in which a marked difference

of approach is implied. Plank is apparently interested in opinions on food qualities which can be expressed in terms of *good* and *bad*, such judgments being of an "overall" nature and very much like those which would be given by consumers. This, as Harries *(6)* and Bate-Smith *(2)* have pointed out, is quite a different approach from that considered when using an "analytical" panel, where the aim is to describe systematically every aspect of, for example, a flavour, without making any prediction of consumer opinion.

Such a procedure may be useful for many purposes, but may well not be helpful where, as is usual, it is important to predict consumer opinion. Ehrenberg and Shewan *(5)* have described experiments in which they found that untrained judges agreed substantially with a trained panel, but for many purposes such a comparison would be the opposite of that required for, ultimately, the trained panel's judgments would have to be "validated" against the opinions of a representative consumer group.

It is true that a consumer group is untrained, but it is just for this reason that overall terms such as *good* and *bad* are used, because they can be easily understood and consumers can readily think and judge in terms of them. It is for this reason that it is not necessary, or even desirable, to try to define *good* to the judges, but that an attempt is made instead to examine the existing or popular interpretation of such terms.

It is worth adding that it is more easily possible to predict consumer judgments from assessments made by a large panel when the latter is made to use descriptive terms such as would be used by a consumer, for it has been shown that there will then often be only a difference of degree between the opinions of the consumers and the experienced judges *(8, 12)*. Nor is it necessarily true that judgments of quality, made in terms of descriptions such as these, must only indicate a judge's preferences, for psychologists and others have often used rating scales based on such terms and have found that a skilled judge can make useful assessments with such a scale, without being influenced to any marked extent by his own preferences.

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FACTORS ASSOCIATED WITH POTATO TEXTURE. II. PECTIC SUBSTANCES

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In an earlier paper *(1)*, the writers explored the relationship of specific gravity and starch content to the texture of cooked potatoes. It will be recalled that both factors displayed a high and statistically significant correlation with organoleptic textural scores. However, in both cases there still remained a certain amount of unexplained variability in the scores of the taste panel.

The role of pectic substances in the architecture of plant cell walls has been well documented *(2, 4, 11, 17)*. Likewise, the invocation of these materials to explain textural relationships in plant tissues has received both theoretical and practical confirmation *(6, 11, 21)*. It has been shown that pectic substances are present in the primary cell wall and that they are also found as a cementing material in the middle lamella between adjoining cells, where they are assumed to be made up principally of calcium salts of pectic and pectinic acids. Since it has been supposed that the differences between soggy and mealy potatoes reflect the extent of cell separation, there have been many attempts to relate potato texture with pectic content *(9, 22, 25, 27)*. On the whole, no obvious relationship has been found.

If a pectic-textural relationship does exist, its apparent absence may be explicable on the following grounds: In the first place, the methods (and conceptual approach) of pectic analysis used by some earlier workers *(7, 25)* are now known to be inadequate. Secondly, it is quite conceivable that gross analyses do not disclose differences in the chemical nature of the various pectic substances in the cell wall and middle lamella. Finally, the relationship itself may be masked by other factors, such as the starch content.

With this view in mind, the present paper deals with a detailed analysis of the various pectic fractions in the identical potato samples, both raw and cooked, used for specific gravity, starch, and organoleptic determinations. Its purpose is to explore the relationship between pectic materials and potato texture and to ascertain the effects of cooking on the chemical constitution of pectic substances.

MATERIALS AND METHODS

Materials. The same 10 samples, of different variety and growing area, were used as were employed in the earlier report *(1)*. Similarly, longitudinal prismatic strips cut from the central region of the tuber served for the analysis of pectic materials. Details of cooking were likewise identical.

Sample preparation. The sample strips (raw and cooked samples) were placed in boiling 95% ethanol, which had been acidified with concentrated HCl to 0.05N. The ratio of alcohol to potato was 2:1. In order to insure complete inactivation of pectic enzymes, the strips were held in the boiling alcohol for 10 minutes. After cooling, the samples, in 63% alcohol, were stored for later analysis. Although this inactivation

treatment was necessary only for raw potatoes, it was carried out with cooked potatoes as well in order to obtain a uniform treatment of the materials.

Extraction of pectic substances. On the basis of McColloch's *(14)* general scheme, the pectic substances were extracted by serial fractionation. The method involved a first extraction with distilled water at room temperature, a second extraction with a calcium-sequestering agent (also at room temperature), and a final extraction with HCl at 85°C.

First extraction. The potato strips in alcohol were homogenized in a Waring blender and then filtered on a Buchner funnel. The pulp was added to twice its weight of distilled water and stirred for 2 hours with a mechanical stirrer at room temperature. After "Hyflo Supercel" was added, the slurry was centrifuged, and the supernatant was collected. The residue was extracted once more in the same way. The second supernatant liquid was added to the first, and the combined extract henceforth called "water-soluble fraction."

Second extraction. The choice of an appropriate calcium-sequestering agent was given some consideration. McColloch *(14)* advocated the use of ammonium oxalate. Since it was desired to determine the calcium content of the pectic material in each fraction, ammonium oxalate could not be used, because calcium would be left in the remaining insoluble fraction in the form of insoluble calcium oxalate.

Owens *et al. (20)* used the sodium salt of ethylene-diamine-tetra-acetic acid (Versene) and sodium hexametaphosphate (Calgon) as calcium-sequestering agents. Since Versene is effective only at a high pH (7.5 and above), there is a possibility of de-esterification and de-polymerization during the extraction. Calgon, on the other hand, is difficult to remove from the alcoholic precipitate, and subsequent gravimetric determinations of pectin, such as the calcium pectate procedure of Carré and Haynes *(5)*, will be in error because of an ash content which is relatively high (up to 50%).[a]

Inasmuch as this high ash content may be reduced (from a maximum of 50% to 15-17%) by extraction at a pH of 5.5 and by subsequent ethanolic precipitation without acidification, a 0.5% Calgon solution was used as the calcium-sequestering agent. Hence the insoluble residue, remaining from the water extraction, was extracted twice with Calgon solutions (pH 5.5), for 2 hours each time, at room temperature. The combined supernatant liquids were designated as the "Calgon-soluble fraction."

Third extraction. The last extraction was performed with 0.05 N HCl at 85°C. for 4 hours. Then the material was centrifuged and the supernatant collected and designated as "HCl-soluble fraction." The centrifugate pulp was also weighed since the pectic substances present here must be added to those in the supernatant. The additional values were computed on the assumption of an equilibrium between filtrate and centrifugate *(20)*.

Analysis of the extracts. Each fraction was treated in the following fashion:

Removal of starch. To avoid erroneously high values, pectic substances must be free of starch before subsequent precipitation by ethanol. This was achieved by enzymic hydrolysis with a solution composed of equal amounts of alpha- and beta-amylase. Neither of these preparations[b] showed any polygalacturonase activity on pectic acid or pectinic acid, as measured by a viscosity test, nor did they show any pectic esterase activity as measured by methoxyl content determination.

The pH of each fraction was adjusted to 5.7, and enzyme solution was added to bring the concentration of the total enzyme to 0.05%. Toluene was put on the surface of the solutions and the enzymes permitted to act for 12 hours. Following starch hydrolysis, the solutions were filtered.

Precipitation of pectic substances by ethanol. The method of Owens *et al. (20)* was followed, except that in order to avoid a high ash content in the precipitate the ethanol was not acidified in the Calgon-soluble fraction. The precipitates were redissolved in hot, distilled water.

Intrinsic viscosity. To the redissolved precipitates NaCl was added to bring the

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[a] Waller and Baker *(26)* have submitted evidence for a chemical union "between phosphorus and pectin" through a reaction involving the orthophosphate ion.

[b] Obtained from the Nutritional Biochemicals Corp.

concentration of the salt to 0.14M. Relative viscosities were determined in an Ostwald-Cannon-Fenske No. 100 viscosimeter at 29.5°C. Three different dilutions of pectic substances were used in each sample. The specific viscosity of each dilution was calculated, and the ratio of specific viscosity to concentration was plotted against concentration on semi-logarithmic paper. The concentrations were obtained from the calcium pectate data. The extrapolation of the curves to 0 concentration gave the intrinsic viscosity.

Calcium content. Calcium content of the pectic substances was calculated from flame-photometric determinations. The instrument used and the method of calibration were described by Brown *et al. (3)*. The samples for this analysis were prepared as follows: An aliquot of the redissolved ethanolic precipitate of the water-soluble fraction was used directly; aliquots of the redissolved ethanolic precipitates of the Calgon- and of the HCl-soluble fractions were combined with their filtrates from the respective fractions. These filtrates were freed from alcohol by evaporation. The volume of the combined solutions was adjusted to be proportional to that of the volume of the original extracts before the precipitation by ethanol. (Combining of the redissolved ethanolic precipitates with the respective filtrates was necessary because calcium appeared in the filtrate as well as in the ethanolic precipitate in both the Calgon- and HCl-soluble fractions.)

The flame-photometric measurements were carried out at 422 mμ with a slit of approximately 0.0075 mm. The blank determination of 0.5% Calgon solution gave a reading which was equivalent to 0.5 p.p.m. calcium.

Methoxyl content. Hinton's method *(10)* was used on an aliquot of the redissolved ethanolic precipitates. A previous determination, using the Owens *et al. (20)* modification of Clark's method, showed that potato pectin of Russet Burbank (Idaho) and Russet Burbank from an unknown source had an acetyl content of 0.02 and 0.04%, respectively. These low values did not justify the use of the more elaborate Shriner *(23)* modification of the Zeisel method. The titration was carried out with a microburette and a Beckman pH meter to an end-point of 7.5. Methoxyl content was calculated in the manner given by Owens *et al. (20)*.

Calcium pectate. Calcium pectate was determined gravimetrically on the same aliquots used in the methoxyl content determination. The method used was given by McColloch *(14)*.

Uronide content. Uronide content was determined in the dried calcium pectate according to the method of McCready *et al. (16)*.

Calcium pectinate. The amount of calcium pectinate is a calculated value. It represents the uronide part of the pectic substances plus the methoxyl and calcium contents. It was calculated in the following manner: uronide content + methoxyl content × 14/31 + calcium content × 19/20. This calculated calcium pectinate content was used in the estimation of the degree of esterification and of the degree of salt form.

EXPERIMENTAL RESULTS

Properties of pectic materials in the water-soluble fraction. The detailed results of analysis of this fraction are presented in Table 1. The uronide content in raw potatoes varies from a high of 23.6 mg./100 g. to a low of 0 mg./100 g. (no pectic substances occur in the water extract of raw Chippewa potatoes). Except in the Bliss Triumph variety, the uronide content and the content of calcium pectinate increase as a result of cooking. In all varieties, the intrinsic viscosity of the pectic materials is lower in the cooked potato than in the raw tuber.

The methoxyl content of these water-soluble pectinates varies from 7.66% to 10.70% (equivalent to 47%-66% esterification) in the raw potatoes. These values, which are rather low in comparison with those of fruit pectins, decrease almost invariably upon cooking. Likewise, the calcium content of the raw tubers is low, varying between 0.24% and 2.38%. There is no consistent relationship of the calcium content with the cooking treatment.

The pectin determined as calcium pectate follows the uronide and the calcium pectinate content. The calcium pectate entity includes the polyuronides, the associated non-uronide polysaccharides, and the precipitating calcium ions. However, because no

TABLE 1

Properties of pectic materials in the cold water extract of potatoes

Variety		Uronide content mg./100g. potato	Methoxyl content mg/100g. potato	Pectin-bound calcium mg/100g. potato	Calcium pectinate mg/100g. potato	Methoxyl content of calcium pectinate in %[1]	Degree of esterification of calcium pectinate[1,2]	Calcium content of calcium pectinate in %[1]	Degree of calcium salt form of calcium pectinate[1,3]	Intrinsic viscosity dl/g.	Calcium pectate mg/100g. potato
Russet Burbank (Utah)	Raw	12.1	1.35	0.19	12.89	10.47	64.19	1.49	14.52	6.12	14.0
	Cooked	52.7	4.93	0.28	55.18	8.93	54.75	0.51	4.97	4.88	75.5
Russet Burbank (Idaho)	Raw	21.3	2.19	0.40	22.67	9.66	59.23	1.76	17.15	9.88	35.0
	Cooked	58.0	5.12	0.31	60.61	8.45	51.81	0.51	4.97	2.84	90.0
Kennebec (Maine)	Raw	10.7	0.87	0.27	11.35	7.66	46.97	2.38	23.20	4.90	13.8
	Cooked	20.6	1.65	0.03	21.39	7.71	47.27	0.14	1.36	2.95	26.4
Green Mountain (Utah)	Raw	20.0	2.15	0.05	21.01	10.20	62.53	0.24	2.34	4.90	22.2
	Cooked	52.3	4.98	0.25	54.79	9.10	55.79	0.45	4.39	3.90	70.5
Cobbler (North Dakota)	Raw	19.6	1.97	0.08	20.57	9.58	58.74	0.39	3.80	4.05	32.4
	Cooked	23.3	2.22	0.06	24.36	9.11	55.86	0.25	2.44	1.57	36.7
Triumph (North Dakota)	Raw	12.4	1.19	0.19	13.12	9.07	55.61	1.45	14.13	9.62	17.7
	Cooked	17.1	1.47	0.17	17.92	8.20	50.27	0.95	9.26	3.80	26.8
Bliss Triumph (Idaho)	Raw	21.2	2.28	0.12	22.34	10.20	62.53	0.54	5.26	2.35	27.6
	Cooked	2.5	0.16	0.10	2.65	6.04	37.03	3.77	36.74	1.90	3.6
White Rose (Calif.)	Raw	23.6	2.40	0.17	24.84	9.66	59.23	0.68	6.63	4.23	45.4
	Cooked	42.0	4.23	0.28	44.17	9.58	58.74	0.63	6.14	3.55	76.2
Red Pontiac (Wisconsin)	Raw	9.5	1.07	0.03	10.00	10.70	65.60	0.30	2.92	2.88	12.3
	Cooked	25.7	2.63	0.03	26.91	9.77	59.90	0.11	1.07	2.67	32.0
Chippewa (Maine)	Raw	None	.....	.....	.....	.....	.....	.....	.....	.....	.....
	Cooked	17.4	1.59	0.04	18.15	8.76	53.71	0.22	2.14	4.75	20.6

[1] Calculated value.
[2] Degree of esterification = 100 × methoxyl content of calcium pectinate/the theoretical maximum methoxyl content (16.25).
[3] Degree of calcium salt form = 100 × calcium content of calcium pectinate/the theoretical maximum calcium content (10.36).

TABLE 2

Properties of pectic materials in the cold Calgon extract of potatoes

Variety		Uronide content mg/100g. potato	Methoxyl content mg/100g. potato	Pectin-bound calcium mg/100g. potato	Calcium pectinate mg/100g. potato	Methoxyl content of calcium pectinate in %[1]	Degree of esterification of calcium pectinate[1,2]	Calcium content of calcium pectinate in %[1]	Degree of calcium salt form of calcium pectinate[1,3]	Intrinsic viscosity dl/g.	Calcium pectate mg/100g. potato
Russet Burbank (Utah)	Raw	38.2	2.38	1.88	41.06	5.80	35.56	4.57	44.54	3.16	48.4
	Cooked	35.3	1.81	2.32	38.32	4.72	28.94	6.05	58.97	1.95	48.6
Russet Burbank (Idaho)	Raw	27.2	1.06	2.11	29.68	3.57	21.88	7.11	69.30	3.65	37.0
	Cooked	33.9	1.21	2.69	37.01	3.27	20.04	7.27	70.86	1.16	47.1
Kennebec (Maine)	Raw	27.7	1.61	1.38	29.75	5.41	33.17	4.64	45.22	3.25	33.7
	Cooked	42.7	2.18	2.40	45.96	4.74	29.06	5.22	50.88	1.85	55.5
Green Mountain (Utah)	Raw	23.5	1.16	1.41	25.36	4.57	28.02	5.55	54.09	2.52	34.2
	Cooked	33.3	1.63	0.95	34.94	4.67	28.63	2.72	26.51	1.38	43.9
Cobbler (North Dakota)	Raw	76.8	5.21	3.42	82.39	6.32	38.75	4.15	40.45	2.10	85.8
	Cooked	71.5	4.31	2.52	75.85	5.68	34.82	3.32	32.36	1.10	83.5
Triumph (North Dakota)	Raw	43.3	1.84	2.68	46.68	3.94	24.16	5.74	55.95	3.32	58.7
	Cooked	55.2	2.22	2.27	58.36	3.80	23.30	3.89	37.91	2.08	73.1
Bliss Triumph (Idaho)	Raw	37.9	1.22	3.39	41.67	2.93	17.96	8.13	79.24	2.10	45.0
	Cooked	86.1	2.63	6.08	93.07	2.83	17.35	6.53	63.65	0.75	106.0
White Rose (Calif.)	Raw	24.4	0.74	2.29	26.91	2.75	16.86	8.51	82.94	3.30	33.1
	Cooked	43.0	1.28	3.41	46.72	2.74	16.80	7.30	71.15	1.67	58.5
Red Pontiac (Wisconsin)	Raw	41.8	2.72	1.19	44.16	6.17	37.82	2.68	26.12	4.40	49.4
	Cooked	34.4	2.21	0.72	36.08	6.12	37.52	2.00	19.49	3.50	44.5
Chippewa (Maine)	Raw	29.9	1.80	1.7¢	31.38	5.74	35.19	5.61	54.68	3.70	37.7
	Cooked	43.2	2.58	1.88	46.16	5.59	34.27	4.07	39.66	2.70	52.0

[1] Calculated value.
[2] Degree of esterification = 100 × methoxyl content of calcium pectinate/the theoretical maximum methoxyl content (16.25).
[3] Degree of calcium salt form = 100 × calcium content of calcium pectinate/the theoretical maximum calcium content (10.26).

ash determination was performed for every sample, the difference between the calcium pectate content and the uronide content is only a rough indication of the non-uronide materials.

Properties of pectic materials in the Calgon-soluble fraction. The purpose of the investigation of this pectic fraction was to gain information concerning pectic materials which are not water-soluble due to their high calcium content. An attempt was also made to identify the site of this fraction within the cell structure. Several thin (10 μ) sections of steamed potatoes were cut with a microtome after paraffin embedding. These were affixed to a glass slide and the paraffin removed by immersion in xylol. After being rehydrated in a graded series of ethanol solutions, the slides were put into a beaker containing distilled water, which was stirred mechanically for 6 hours. The slides were then placed in ruthenium red solution. Under the microscope, a pink coloration was clearly seen in the cell walls and middle lamella. Slides treated with Calgon were scarcely stained at all, indicating that most of the pectic material on the surface of the sections was extracted. Although ruthenium red is nonspecific, it may be suggested that the pectic substances removed by the Calgon came from the exposed surfaces of the middle lamella and of the cell wall. The application of a new staining method of McCready and Reeve[6] *(15)*, based on ester groups and claimed to be specific for pectin in plant tissues, was not successful due to the low methoxyl content of the pectic substances in potatoes.

The chemical characterization of the Calgon-soluble fraction is given in Table 2. In some cases, the amounts of calcium pectinate and uronide in this fraction are 5 and 6 times greater than the amounts in the water-soluble fraction. During cooking, these entities may increase or decrease. The methoxyl content of this fraction is quite low: 16%-39% esterification, agreeing with results on tomato *(12, 13)* but not on apple *(8)*. As in the water-soluble fraction, this methoxyl content also decreases upon cooking.

The calcium content of the calcium pectinate is higher in the Calgon-soluble fraction than in the water-soluble fraction. (This probably explains the insolubility of the former in water.) In most cases the calcium content of the pectinate in the Calgon-soluble fraction decreases during cooking. The intrinsic viscosity of this fraction is much lower than that of the water-soluble fraction. Like the latter, it decreases in each variety as a result of cooking.

The ash content of the calcium pectate of the Calgon fraction is 15-17%, which is in the range of values found by Newbold and Joslyn *(18)* in calcium pectates from citrus pectin. However, these ash contents are higher than those found in the calcium pectates of the water- and HCl-soluble fractions of pectic substances in potatoes (7-9%), indicating that polyphosphates are adsorbed in the calcium pectate of the Calgon-soluble fraction.

Properties of pectic materials in the HCl-soluble fraction. It is evident from Table 3 that there are some significant differences between this fraction and the two previously considered. Hence, there is probably some justification for a separate treatment of its pectic materials. The uronide and calcium pectinate contents are from 3 to 5 times as great as those of the Calgon-soluble fraction. Thus, the major part of the potato pectins are to be found in this fraction. In all varieties, a large decrease occurs in the calcium pectinate content during cooking.

Although the methoxyl content approaches that of the Calgon-soluble fraction, it is often somewhat lower (perhaps as a result of de-esterification during the extraction). In cooked potatoes, the methoxyl content is lower than in raw ones. Another interesting characteristic is the very high calcium content, which occasionally (in combination with the methoxyl groups) accounts for more than the theoretically expected carboxyl groups. The discrepancies possibly are due to experimental errors.

Intrinsic viscosity values are mostly higher than those of the Calgon-soluble fraction. Usually, only a slight decrease occurs in this value following cooking; occasionally, an increase is found. More non-uronide material appears to be present in the calcium pectate of this fraction than in the other two. This additional material may be derived from other water-insoluble polysaccharides during the HCl extraction.

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[6] We wish to acknowledge, with thanks, the kind permission to use this technique in advance of formal publication.

TABLE 3

Properties of pectic materials in the hot hydrochloric acid extract of potatoes

Variety		Uronide content mg/100g. potato	Methoxyl content mg/100g. potato	Pectin-bound calcium mg/100g. potato	Calcium pectinate mg/100g. potato	Methoxyl content of calcium pectinate in %[1]	Degree of esterification of calcium pectinate[1, 2]	Calcium content of calcium pectinate in %[1]	Degree of calcium salt form of calcium pectinate[1, 3]	Intrinsic viscosity dl/g.	Calcium pectate mg/100g. potato
Russet Burbank (Utah)	Raw	116.7	4.94	4.41	124.11	3.98	24.40	3.55	34.60	4.03	236.0
	Cooked	68.6	2.82	2.82	72.55	3.89	23.85	3.89	37.91	2.04	144.0
Russet Burbank (Idaho)	Raw	130.2	4.05	13.59	144.95	2.79	17.10	9.38	91.42	3.50	338.7
	Cooked	53.2	1.64	5.46	59.22	2.77	16.98	9.22	89.86	2.36	114.2
Kennebec (Maine)	Raw	93.5	1.99	8.48	102.46	1.94	11.89	8.28	80.70	3.95	212.5
	Cooked	67.9	1.06	7.55	75.56	1.40	8.58	9.99	97.36	3.70	112.2
Green Mountain (Utah)	Raw	121.2	5.79	4.92	128.48	4.51	27.65	3.83	37.32	3.10	268.6
	Cooked	55.8	2.35	3.55	60.24	3.90	23.91	5.89	57.41	3.21	110.8
Cobbler (North Dakota)	Raw	206.5	6.56	8.38	217.43	3.02	18.51	3.85	37.52	1.90	262.5
	Cooked	147.1	5.83	7.68	157.04	3.71	22.75	4.89	47.66	4.65	206.0
Triumph (North Dakota)	Raw	144.6	3.14	16.52	161.72	1.94	11.89	10.21	99.51	3.55	323.1
	Cooked	109.6	2.22	7.06	117.30	1.89	11.59	6.02	58.68	1.10	290.0
Bliss Triumph (Idaho)	Raw	210.5	11.07	8.95	224.00	4.94	30.29	3.99	38.88	2.50	282.5
	Cooked	46.4	1.47	3.77	50.64	2.90	17.78	7.44	72.51	4.45	69.8
White Rose (Calif.)	Raw	133.7	5.86	8.84	144.15	4.07	24.95	6.13	59.74	6.82	263.8
	Cooked	89.5	2.91	9.02	99.38	2.93	17.96	9.08	88.50	3.40	179.0
Red Pontiac (Wisconsin)	Raw	118.6	7.08	4.09	125.68	5.63	34.51	3.25	31.68	3.77	315.2
	Cooked	103.1	5.66	2.83	108.24	5.23	32.07	2.61	25.44	3.14	225.3
Chippewa (Maine)	Raw	114.5	4.73	6.15	122.48	3.86	23.67	5.02	48.92	4.75	168.2
	Cooked	82.2	3.26	5.21	88.61	3.68	22.56	5.88	56.31	3.30	135.0

[1] Calculated value.
[2] Degree of esterification = 100 × methoxyl content of calcium pectinate/the theoretical maximum methoxyl content (16.25).
[3] Degree of calcium salt form = 100 × calcium content of calcium pectinate/the theoretical maximum calcium content (10.26).

TABLE 4

Properties of total pectic substances in potatoes.

Variety		Organoleptic score	Uronide content mg/100g. potato	Calcium content mg/100g. potato[1]	Calcium pectinate content mg/100g. potato	Calcium pectate content mg/100g. potato
Russet Burbank (Utah)	Raw		167.0	6.47	179.1	298.4
	Cooked	194	156.6	5.42	166.1	268.1
Russet Burbank (Idaho)	Raw		178.7	16.10	197.3	410.7
	Cooked	183	145.1	8.46	156.8	251.3
Kennebec (Maine)	Raw		131.9	10.13	143.6	260.0
	Cooked	169	131.2	9.98	142.9	194.1
Green Mountain (Utah)	Raw		164.7	6.38	174.8	325.0
	Cooked	168	141.4	4.76	150.0	225.2
Cobbler (North Dakota)	Raw		302.9	11.88	320.4	380.7
	Cooked	162	241.9	10.26	257.3	326.2
Triumph (North Dakota)	Raw		200.3	19.41	221.5	399.5
	Cooked	130	181.9	9.50	183.6	389.9
Bliss Triumph (Idaho)	Raw		269.6	12.45	288.0	355.1
	Cooked	115	135.0	9.95	146.4	179.4
White Rose (Calif.)	Raw		181.7	11.30	195.9	342.3
	Cooked	112	174.5	12.71	190.3	313.7
Red Pontiac (Wisconsin)	Raw		169.9	5.32	179.8	376.9
	Cooked	107	163.2	3.58	171.2	301.8
Chippewa (Maine)	Raw		144.4	7.91	153.9	205.9
	Cooked	102	142.8	7.13	152.9	207.6

[1] Calcium associated with pectic substances.

Relationship of pectic materials to potato texture. In consideration of the role of pectic materials as cementing substances in plant tissues, regression coefficients were calculated for various pectic fractions with texture as determined organoleptically. To this end, certain properties of the total pectic materials were determined (*i.e.*, the sums of the 3 fractions), and these are presented in Table 4.

The total calcium pectinate content varies from 143.6 to 320.4 mg./100 g. fresh potato in raw tubers, and this amount decreases during cooking. Due to the partial de-esterification and de-polymerization which occur during extraction with hot acid, neither the total methoxyl content nor the average intrinsic viscosity is given. (It is particularly to be recalled that the HCl-soluble fraction yields the main portion of the pectic material.) The amount of calcium pectate occasionally may be double that of the calcium pectinate. The former is presented mainly for comparisons with older results, in which this determination was employed.

When the total calcium pectinate is compared with the texture scores, the "r" value is 0.021. On the basis of the total calcium associated with pectic materials, the regression coefficient is −0.151. In view of the possibility that the Calgon-soluble fraction might represent more nearly the pectic materials of the middle lamella, some likely factors from this fraction were computed. However, the "r" value for per-

centage of calcium in the calcium pectinate is also low: 0.178. In the case of the intrinsic viscosity of the pectinate of this fraction, "r" is found to be −0.403. No values in the water-soluble fraction appear to have any relationship with the texture scores.

DISCUSSION

It is of interest to consider, first of all, the nature of the 3 fractions extracted here from the parent pectic material in the potato. The water-soluble fraction, constituting a minor portion of the pectic material, is characterized by a relatively high methoxyl content, low calcium content, and a high intrinsic viscosity.[4] Being soluble, this fraction can contribute little to any cementing effect.

There are certain indications that the Calgon-soluble fraction is derived, at least in part, from the middle lamella. First of all, there is its relatively high calcium content, which accords with the observations of other workers *(2, 17)* that the middle lamella pectin is rich in calcium. Secondly, there is the well-known macerating effect *(21)* which results from the application of calcium-sequestering agents to plant tissues. Thirdly, staining experiments in conjunction with Calgon-application appear to support the view that pectic materials are removed thereby from both the middle lamella and the cell wall. Possibly the low intrinsic viscosity in this fraction is a result of the mechanism of solvent action *(i.e.*, since the large metaphosphate polymers of Calgon cannot penetrate deeply into the matrix of insoluble polysaccharides, only molecules exposed at surfaces may be brought into solution).

The hot acid extraction will remove the remainder of the more deeply located, insoluble pectic substances. This is effected by solubilization of the calcium and other insoluble salts, breakage of hydrogen bonds (accompanied also by breakage of ester bonds), and breakage of primary valence bonds. The resulting depolymerization from the last-mentioned effect presumably results in a greatly lowered intrinsic viscosity. Since this value is somewhat higher than that in the Calgon fraction, it is possible to infer that this fraction is composed of molecules of originally high molecular weight. Part of the abundant calcium in this fraction may be present in an adsorbed form on the pectic matrix.

According to McColloch *(14)*, treatment with 0.05 N HCl will solubilize and therefore remove the inorganic salts, including calcium. The remaining calcium, therefore, should be in an "associated" form with the pectic substances. In one case, Chippewa, the amount of calcium removed by the preliminary treatment (with 0.05 N HCl in alcohol and in the filtrate of the ethanolic precipitation of the water-soluble fraction) was measured and found to be 18.23 mg. calcium in 100 g. cooked potatoes, on a fresh weight basis. Since the total calcium associated with the 3 fractions of pectic substances from the corresponding sample was 7.13 mg. in 100 g. potato, between one-third and one-fourth of the total calcium was in the "associated" form. As has been mentioned above, this associated calcium is not necessarily combined chemically with the pectic substances.

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[4] Intrinsic viscosity is an index of the weight average molecular weight and varies directly as a power function of the latter *(19, 24)*.

It should be stressed that the 3 fractions analyzed here are neither actually nor conceptually discrete chemical entities. The dividing lines are quite arbitrarily drawn on the basis of solubility differences.

In general, cooking has major effects on the pectic materials of the potato. The most obvious is a solubilization effect. Pectic materials become more soluble as a result of de-polymerization, breakage of hydrogen bonds, and higher temperature of solution. Hence, there is an increase in the pectic content of the water-soluble fraction (and occasionally in that of the Calgon-soluble fraction), with an invariable decrease in that of the HCl-soluble fraction (often also in that of the Calgon-soluble fraction). There is also a decrease in the calcium content of the cooked potatoes and a decrease in intrinsic viscosity. Much of the solubilized pectic substance diffuses into the cooking medium.

A constant effect, found in all fractions, is a lower methoxyl content in the cooked potatoes. This may be due to the activity of enzymes in the first few minutes of heating, perhaps to a direct heat-induced de-esterification, or to removal of fractions of higher ester content by the cooking water. There is some controversy in the literature *(11)* concerning the possibility that a temperature increase alone may be responsible for de-esterification.

The determination of "calcium pectate" perhaps is only of historical interest in work on potato pectins. In the first place, values may be erroneously high because of the presence of starch in the precipitate. Thus, Sweetman *(25)* found 3.2%-5.4% total pectic material on a dry weight basis. Freeman and Ritchie *(9)*, who used ptyalin to remove starch, obtained 1.8-3.3% total pectic substances on a dry weight basis. The values here, recomputed on this basis, are 1.1%-2.1%—possibly due to the higher activity of the pure amylases. Nevertheless, even these values may be almost double the calcium pectinate content determined from the uronide material. There are obviously other associated polysaccharides and ash which may be entrapped in the meshwork of pectic material, which together can give spurious values when determined as "calcium pectate."

It is important to note that there is no obviously direct relationship between the characteristics of pectic materials and potato texture. The highest gross coefficient was found between texture and the intrinsic viscosity of the pectinate of the Calgon-soluble fraction ($r = -0.403$). These results would seem to negate the supposition that cell separation as such is responsible for mealy texture and cell adhesion responsible for soggy texture. Further work to clarify this problem will be reported subsequently.

SUMMARY

Ten potato varieties were compared on the basis of a detailed analysis of pectic materials in the raw and cooked condition. Extraction was performed successively with three agents: cold water; cold Calgon; hot dilute HCl. The analysis of each of these three fractions was employed to elucidate the nature of pectic substances in potatoes and the effects of cooking.

Most of the pectic material was found in the HCl-soluble fraction, and in decreasing order, in the Calgon- and water-soluble fractions. The water-soluble fraction was characterized by the highest intrinsic viscosity and highest methoxyl content. The intrinsic viscosity of the HCl-soluble frac-

tion was higher than that of the Calgon-soluble fraction and was probably much higher initially (before acid hydrolysis). The greatest amount of calcium was found in the HCl-soluble fraction, and the next-greatest in the Calgon-soluble fraction.

In virtually all cases cooking caused a decrease in intrinsic viscosity, in calcium content, and in methoxyl content. It caused an increase in solubility of the pectic materials, resulting particularly in a higher pectic content of the water-soluble fraction following cooking.

No obviously direct relationship was found between the various fractions of pectic substances and potato texture. Various aspects of the extraction procedure and the significance of the results were discussed.

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FACTORS ASSOCIATED WITH POTATO TEXTURE. III. PHYSICAL ATTRIBUTES AND GENERAL CONCLUSIONS

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The writers' previous studies on starch content *(2)* and pectic materials *(3)* did not, in themselves, fully clarify the relationship of the texture of cooked potatoes with various chemical attributes. Consequently, the question arises whether differences in texture are related to differences either in the ease with which the cells can be separated or in the actual extent of cell separation in the tissues of the cooked potatoes.

Various approaches to the problem of estimating the extent of intercellular spaces are possible. The most direct approach is a microscopic study of the tissues involved, as exemplified in the investigations of Reeve *(13)* and Personius and Sharp *(9)*. However, because of the difficulty of cutting and handling the loose, cooked tissue, the study of internal spaces has also been attempted indirectly. The ingenious determinations of tensile strength by Personius and Sharp *(7)* measure "cell adhesion" in cooked tissue and thereby the strength of the intercellular cementing bond. Presumably, where the bond is weaker, one could suppose that the cells are more likely to be separated easily.

Similarly, the degree of sloughing (disintegration by fragmentation during boiling) may be a clue to the ease of cell separation. Thus, Sweetman *(15)* and Barmore *(1)* have reported a correlation between sloughing and mealiness and between sloughing and starch content. Likewise, Whittenberger *(16)* and Whittenberger and Nutting *(17)* found a general correlation between specific gravity and sloughing. In addition, Pyke and Johnson *(11)* showed that as calcium ion was added to the cooking water, the incidence of sloughing declined, thus indicating that the firming of the pectic materials of the middle lamella had increased the strength of the intercellular bond. This finding confirmed the results of earlier work by Personius and Sharp *(8)*.

Freeman *(4)* made physical measurements of the volume of internal pores in dried slices of baked potato tissue. The resulting data were found to be significantly correlated with the organoleptic scores for texture. Thus, when soggy tissue was dried, the slice became a dense and horny vitreous sheet. On the other hand, mealy tissue produced a loosely porous mass.

In the present study, cooked and raw potato tissues have been subjected to mechanical tests to determine the degree of cell adhesion; the amount of sloughing has been ascertained; and the cooked tissues of different potato varieties have been analyzed microscopically. In a general conclusion, multiple correlations have been computed for pertinent data, and the overall results evaluated.

MATERIALS AND METHODS

Materials. The first paper *(2)* of this series presents a detailed account of the samples used and the method of sample preparation and cooking.

Tensile strength determination. The measurements were carried out with an apparatus similar to that of Personius and Sharp *(7)*. Radial-longitudinal slabs were cut out from the central portion of each raw tuber by a fixed razor. These slabs were then cut by die to the final shape and the dimensions at the narrow "waist" measured by micrometer. Traction was applied by water running to a suspended bucket. The weight of bucket, contents, and holder in grams, divided by the cross-sectional area of the original piece in square centimeters, was indicated as the tensile strength. Preliminary tests with the Russet Burbank (Idaho) variety showed that 11 measurements were needed in order to insure a 98% probability that the mean would be within ± 150 g. of the true mean. No measurements were carried out on cooked potatoes because of the weakness of the tissues.

Shear strength determination. Rectangular prisms were cut from the medullary region of the tuber, parallel to the long axis of the latter. In raw potatoes, the cross-sectional dimensions of these prisms were 5 × 5 mm.; in the case of cooked potatoes, 10 × 10 mm. The measurements were carried out in a guillotine-type apparatus described

TABLE 1

Physical attributes of raw and cooked potatoes

Variety		Tensile strength g/cm²	Shear strength g/cm²	Sloughing per cent	Cell separation per cent[1]
Russet Burbank (Utah)	r[2]	9,093	10,814		
	c[3]		314	64.24	93.2
Russet Burbank (Idaho)	r	7,274	8,313		
	c		365	22.71	77.2
Kennebec (Maine)	r	7,070	7,862		
	c		379	11.22	82.0
Green Mountain (Utah)	r	7,499	7,765		
	c		212	61.69	81.2
Cobbler (N. Dakota)	r	7,867	8,934		
	c		302	30.12	53.2
Triumph (N. Dakota)	r	7,327	8,997		
	c		309	7.54	22.8
Bliss Triumph (Idaho)	r	5,730	6,420		
	c		174	8.82	52.0
White Rose (California)	r	6,856	9,188		
	c		333	11.18	41.6
Red Pontiac (Wisconsin)	r	7,727	9,076		
	c		376	25.83	64.8
Chippewa (Maine)	r	6,370	7,389		
	c		342	15.66	55.6

[1] Intercellular spaces per 100 cell interstices counted.
[2] r = raw.
[3] c = cooked.

by Pitman *(10)*. The shear strength is the stress (in grams per square centimeter) required to rupture these prisms on two parallel faces, perpendicular to the long axis of the prism. Samples were chosen from the same potatoes which served for the tensile strength measurement. For measurements on cooked potatoes, the prisms were transferred to a water bath containing distilled water at 35°C. following the usual 10 minutes of boiling. This was necessary because it was found that surface-toughening occurred on standing in air, thus increasing the stress required to cause rupture.

Per cent sloughing. Following the method of Whittenberger and Nutting *(17)* twenty 10-gram cubes were prepared from each variety, each cube being cut from the central part of a single tuber. These were boiled for 30 minutes. Per cent sloughing loss was calculated on a dry weight basis.

Histological examination. Raw and cooked potatoes, including ruptured specimens from the mechanical tests, were fixed in a chromic acid-acetic acid-formaldehyde mixture *(5)* to preserve and harden the cell structures. After gradual dehydration, the material was infiltrated with paraffin and cut at a thickness of 10 μ. Iron-haematoxylin was the principal histological stain. Rawlins *(12)* believes that the absorption of ferric salts is fairly specific for the pectic substances of the middle lamella. [Also see Johansen *(5)*]. In the cooked tissues, the number of open intercellular spaces per 1,000 randomly chosen cell interstices were counted for each variety, with particular avoidance of regions of obvious tissue rupture. Results were expressed as per cent cell separation.

RESULTS AND DISCUSSION

The results of the various physical measurements are presented in Table 1. It is apparent that the values for tensile strength and shear strength are unrelated to potato texture. (The varieties are ranked in order of decreasing mealiness. Organoleptic scores are presented in Tables 2 and 4.) In the case of the raw potatoes, there is an adequate microscopic explanation for the absence of a mechanical strength-textural relationship: Samples from the raw strength test were characterized by broken cell walls at the rupture surface. Thus, not the strength of the intercellular cement but the strength of the cell wall is being measured in the raw potato.

The absence of a correlation between shear strength of the cooked sample and texture probably indicates that the property of mealiness or sogginess depends on factors other than the gross mechanical strength of the intercellular bond. It is of interest to note that similarly Personius

TABLE 2

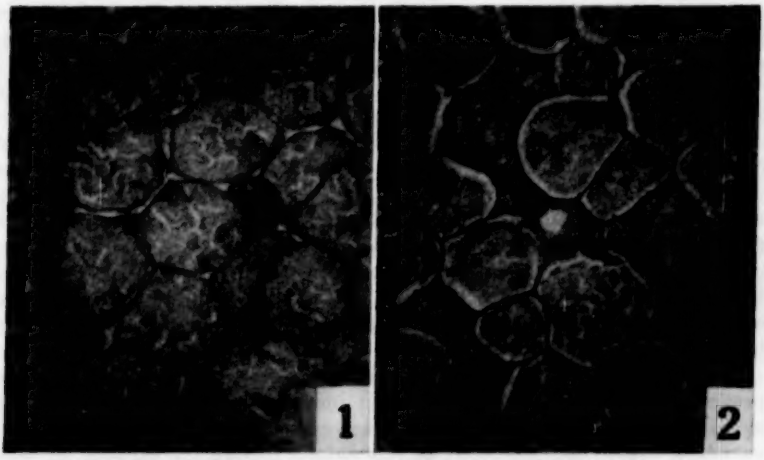
Data used in calculation of first multiple correlation

Variety	1 Total taste panel score	2 % total starch[1]	3 % of calcium of pectinate in Calgon fraction[1]	4 Intrinsic viscosity of pectinate in Calgon fraction dl/g[1]
Russet Burbank (Utah)	194	15.92	6.05	1.95
Russet Burbank (Idaho)	183	15.85	7.27	1.16
Kennebec	169	15.21	5.22	1.85
Green Mountain	168	11.40	2.72	1.38
Cobbler	162	9.42	3.32	1.10
Triumph	130	8.55	3.89	2.08
Bliss Triumph	115	8.42	6.53	0.75
White Rose	112	9.70	7.30	1.67
Red Pontiac	107	6.25	2.00	3.50
Chippewa	102	7.75	4.07	2.70

[1] In cooked potatoes.

and Sharp *(7)* found "no difference between soggy and mealy potatoes in the adhesion of cells of the raw or cooked tissue." It may be mentioned, in this connection, that mechanical tests in cooked samples were all characterized distinctly by *separation* of intact cells at the rupture surface.

When sloughing measurements were made, the values were roughly grouped, indicating that more sloughing occurs in the mealier varieties. Computation of a regression coefficient with organoleptic scores gives an "r" value of 0.62, which is reasonably high but, in view of the small number of samples, not statistically significant. Unfortunately, the Whittenberger and Nutting method *(17)* not only measures losses by physical disintegration but also losses by the diffusion of starch and various solutes. The relatively high value of the coefficient is especially surprising also in that *(1)* a ten-gram cube often includes extra-medullary tissues and that *(2)* the time of boiling is three times as long as that used for all the other data in the study.



Cooked potatoes, stained with iron haematoxylin. ×170.

Figure 1. Section of cooked sample of Russet Burbank potato from Utah. Note extent of cell separation, indicated by intercellular spaces.

Figure 2. Section of cooked sample of Chippewa potato from Maine. Scarcely any intercellular spaces have been formed among the cells.

Figure 1 is a representative microscopic section from a cooked sample of the Russet Burbank (Utah) variety. Figure 2 is from a cooked sample of the Chippewa (Maine) variety. As representatives of the extremes in texture, these varieties also show a major point of difference microscopically. It will be noted that cells are extensively separated in the Russet Burbank potato, and the middle lamella is not at all clearly defined. In the Chippewa variety (following the identical treatment) the cells are tightly adherent and the middle lamella and primary cell wall stain deeply.

In essence, this difference holds between the mealy and the soggy

groups of tubers. (Raw tubers of each variety lack noticeable intercellular spaces.) The coefficient of regression between per cent cell separation and texture score is fairly high, 0.69, even though the former value is obtained by the very arbitrary and approximate basis of counting noticeable random intercellular spaces in the slides of each sample. These two features are therefore apparently related, even though the relatively few samples preclude statistical significance for this "r" value.

The strong suggestion of the accumulated evidence that cell separation, and hence degraded pectic material, is involved in determining potato texture leads logically to a re-examination of the pectic data. If the data for those pectic materials reasonably assumed to be related to the strength of the intercellular bond (per cent calcium and intrinsic viscosity in the pectinate of the Calgon-soluble fraction) are placed side by side with the data for starch content and organoleptic scores, an interesting relation becomes apparent (Table 2). On close inspection it will be noted that where the starch content and the texture scores do not change in the same degree, a definite change occurs in the values of the pectic properties. The obvious suggestion of an interrelationship was checked by the computation of partial and multiple coefficients of correlation.

Considering the organoleptic score as the dependent variable and the other three factors in Table 2 as independent variables, it may be seen that the values of the coefficients continually increase, as they are followed from the gross coefficient to the first and second order partial coefficients (Table 3)[1]. This constant change in values indicates an interrelationship among the three independent variables.[2] It will also be noted that although the starch-texture coefficients are positive (directly related), the pectic-texture coefficients are negative (inversely related). Based on the combined factors, the multiple coefficient is 0.97. This value is highly significant and indicates that the three factors here can explain 94% of the variation in texture among the potato samples.

When total calcium associated with pectic material and total calcium pectinate are considered as the pectic independent variables (Table 4), again a strong interrelationship is shown among the three independent variables (Table 5). However, in this case, although the calcium content-texture coefficients are again negative, the total calcium pectinate-texture

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[1] In calculating partial coefficients, three assumptions have been made: (1) the gross correlations have linear regressions; (2) each independent variable has an effect on the dependent variable (*i.e.*, the texture score); and (3) these effects are additive.

The gross coefficient shows the relationship between the dependent variable and an independent variable, including the effects of other independent variables on the latter (interrelationship). The partial coefficient shows the same relationship after the removal of the effects of one (first order) or two (second order) of these other variables.

If there is little or no difference in value between the gross and the partial coefficients, a persistent relationship is indicated between the independent variable and the dependent one, with little interrelationship between the particular independent variables involved. If there is a difference, an interrelationship between the independent variables may be assumed *(6)*.

[2] The change in sign (from positive to negative) for the pectic factors is an additional strong indication of interrelationship.

TABLE 3

Coefficients of correlation (based on Table 2)

Gross coefficients[1]	First order partial coefficients[1]	Second order partial coefficients[1]	Multiple coefficient[1]
r_{12} 0.8834	$r_{12.3}$ 0.9368	$r_{12.34}$ 0.9614	
	$r_{13.2}$ −0.6776		
r_{13} 0.1781		$r_{23.24}$ −0.7469	$R_{1.234}$ 0.9727
	$r_{13.4}$ −0.0314		
	$r_{14.2}$ −0.0190		
r_{14} −0.4029		$r_{14.23}$ −0.4018	
	$r_{14.3}$ −0.3685		

[1] Subscripts 1,2,3,4, refer respectively to columns in Table 2. See text for explanation of coefficients.

coefficients are *positive*. Here the multiple coefficient is 0.96, and again it is highly significant. About 92% of the variation of the texture scores can be explained by the variation of these three independent factors.

When the microscopic results and statistical analyses of the present study are considered against the background of the various investigations on potato texture, several broad conclusions are clearly indicated:

a. The starch content is the principal determining factor in potato texture. As suggested by many workers, its probable role in the development of texture is to bring about a slight distension of the cell walls during gelatinization, inducing a tendency in the cells to round off from each other.

b. Cell *separation* is the principal physical attribute of a mealy potato, and the degree of cell separation is a measure of the degree of mealiness (or sogginess). This is shown very interestingly by Reeve *(11)* in his discussion of the "popcorn" effect in dehydrated potato cubes and by the microscopic study here.

c. Certain characteristics of the pectic materials play a role in the

TABLE 4

Data used in calculation of second multiple correlation

	1	2	3	4
Variety	Total taste panel score	% total starch[1]	Total calcium associated with pectic material mg/100g[1]	Total calcium pectinate mg/100g[1]
Russet Burbank (Utah)	194	15.92	5.42	166.1
Russet Burbank (Idaho)	183	15.85	8.46	156.8
Kennebec	169	15.21	9.98	142.9
Green Mountain	168	11.40	4.76	150.0
Cobbler	162	9.42	10.26	257.3
Triumph	130	8.55	9.50	183.6
Bliss Triumph	115	8.42	9.95	146.4
White Rose	112	9.70	12.71	190.3
Red Pontiac	107	6.25	3.58	171.2
Chippewa	102	7.75	7.13	152.9

[1] In cooked potatoes.

TABLE 5

Coefficients of correlation (based on Table 4)

Gross coefficients[1]	First order partial coefficients[1]	Second order partial coefficients[1]	Multiple coefficient[1]
r_{12} 0.8834	$r_{12.3}$ 0.8962	$r_{12.34}$ 0.9603	
	$r_{13.2}$ −0.3526		
r_{13} −0.1508		$r_{23.24}$ −0.7101	$R_{1.234}$ 0.9615
	$r_{13.4}$ −0.1661		
	$r_{14.2}$ 0.5754		
r_{14} 0.0205		$r_{14.23}$ 0.7881	
	$r_{14.3}$ 0.0735		

[1] Subscripts 1,2,3,4, refer respectively to columns in Table 4. See text for explanation of coefficients.

development of a textural quality. Mainly, their effect is opposed to that of the starch. Where the starch tends to cause cell rounding and separation, these characteristics of the pectic materials counterpose an adhesive force tending to prevent cell separation. In this respect, Whittenberger *(16)* has assigned a "passive" rôle to the pectic materials. However, it is clear that the textural effect of calcium content and molecular weight in the pectic substances is essentially *negative.*

The positive relationship of total calcium pectinate with textural quality may be explicable *via* the collodial properties of the pectic substances. Generally, pectic materials will tend to swell in hot water as they become hydrated. This would tend to produce enlargement of the middle lamella and greater cell separation. However, the tendency could be counterposed by cationic (*e.g.*, calcium) links between adjacent pectin molecules, causing them to be held together. (Thus would be explained the negative regression coefficient between calcium content and texture.)

As a final point, it should again be stressed that the specific results of this study should not be taken as indicative of general varietal quality in potatoes or even of varietal quality in a certain area of growth. Rather, textural quality is related to general chemical properties of the potato tissues. It is only in this sense that specificity of the results is suggested.

SUMMARY

Measurements of strength (tensile and shear) in raw and cooked potatoes showed no definite relationship with organoleptic scores of texture of the cooked product. Rupture in raw potatoes occurred across the cell walls, whereas in cooked potatoes rupture took place in the middle lamella between intact cells.

The amount of sloughing was definitely correlated with texture scores ($r = 0.62$). Microscopic examination of potato tissues showed that in the mealier potatoes, cells were separated from each other to a greater extent than in the soggier ones.

Evaluation of partial and multiple correlation coefficients indicated that starch content and various pectic characteristics were interrelated. Variations in the starch content, in combination with variations in the

calcium content and in the intrinsic viscosity of the Calgon-soluble fraction of the pectic materials, accounted for 94% of the variation of texture scores. Variations in combined starch content, total pectic calcium, and total pectinate could account for 92% of the variation of textural quality. Both values are highly significant statistically. Therefore, it is suggested that in the development of potato texture the swelling of the gelatinized starch is the major factor tending to cause rounding-off of cells and thus cell separation. This tendency for cell separation is counterposed to a lesser extent by the calcium content and molecular size of the pectic materials in the middle lamella and cell wall.

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MAPLE SIRUP. VIII. A SIMPLE AND RAPID TEST FOR THE ANALYSIS OF MAPLE SIRUP FOR INVERT SUGAR[a,b]

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A simple, rapid threshold test for the approximate analysis of invert sugar in maple sirup is described. The method is based on the selection of sample dilutions such that, for any given percentage of invert, one dilution will give a positive color test while the adjacent higher dilution will give a negative test reaction. The test requires only a simple kit, obtainable from all drug stores, and such equipment as can be found in the home or processing plant.

Many farmer-producers of maple products extend the income from their maple crop by making and selling a number of maple confections in addition to maple sirup. One of these confections is a maple cream or "butter" (a fondant type of spread) which consists of a myriad of microscopic sugar (sucrose) crystals in sirup. The product, like many of the "butters," can be spread on bread, toast, pancakes, and other food. The process used in making this maple spread is relatively simple. Ordinary commercial sirup is boiled until sufficient water has been evaporated to give a sugar solids concentration that will elevate the boiling point approximately 20°F. above that normal for boiling water. To prevent growth of large crystals the mass is rapidly cooled to about 100°F. and then stirred, either by hand or with a mechanical mixer, until the sucrose crystallizes to a cream-like consistency.

To obtain creams of the proper consistency the amount of simple sugars (invert sugar) must be within certain limits. When no invert sugar is present and no provision is made to form it during the preparation of the product, the cream may have an undesirable, coarse, gritty crystalline structure. However, this condition seldom is encountered. On the other hand, if a large amount of invert sugar is present no crystallization of the sucrose will occur. If the amount of invert sugar is only slightly in excess of optimum the cream may be too fluid and will separate on storage, producing a product of unpleasant appearance. Just what the optimum level of invert sugar is, is governed by a number of conditions such as pH and concentration of solids (boiling point elevation). The amount of invert which can be tolerated is not critical within a range of 0.5 to 2%. Somewhat larger amounts of invert (2 to 4 per cent) can be tolerated providing the temperature of the boiling sirup is increased by 2° to 4° above the normal

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[a] Presented at the Fourteenth Annual Meeting of the Institute of Food Technologists, Los Angeles, California, June 27-July 2, 1954.

[b] Maple Sirup VII was the article by Porter, W. L., Hoban, Nancy, and Willits, C. O., entitled "Contribution to the Carbohydrate Chemistry of Maple Sap and Sirup," *Food Research*, 19, 597-602 (1954).

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of 20°F. The amount that the temperature must be increased will depend on the amount of invert sugar present. This, however, necessitates that each batch of sirup must be tested in small lots (by trial and error) prior to making the large batches of cream.

A simpler procedure would be the use of maple sirup which has an invert sugar concentration which falls within limits fairly close to the optimum (0.5 to 2%) so that standard cooking temperatures and process can be used. This procedure would require a chemical analysis of the sirups. Unfortunately, most producers do not have available on the farm the facilities necessary for such determinations, and cannot afford to have the analyses made by a commercial analytical laboratory. This paper reports a simple method for an approximate analysis for invert sugar in maple sirup, and which is effective on sirups containing up to 7% of invert sugar.

TEST FOR INVERT SUGAR

The test to be described is a simple, inexpensive, chemical method of analysis which may be used to obtain an approximation within 1% of the invert content of maple sirup. It is best applied as a limiting test by which a sirup can be shown to contain more than or less than a certain percentage of invert sugar. The test is based on the use of a simple reagent[4] which was developed for the determination of sugar in the urine of diabetic patients and is obtainable at any drug store. In addition to this reagent, medicine droppers, and test tubes, there is required only such equipment as is found in the home.

For making maple cream or "butter" there is no one percentage of invert sugar concentration that will be optimum under all conditions and for all recipes. Usually the best results are obtained if the sirup contains not more than 2% of invert sugar.

Apparatus:

1 transparent measuring cup
1 quart measure (milk bottle or fruit jar can be used)
1 container for mixing (sap-gathering pail, 16 to 20 qt. size)
1 long handled spoon or paddle
5 clean, dry, 4-oz. tumblers
1 bottle of "Clinitest"[4] tablets with color chart
6 test tubes (approx. ½ inch diameter)
1 test tube holder*
6 medicine droppers (for use with "Clinitest" tablet)

* Made from a wooden block approximately 1 x 3 x 8 in. with a line of 5 holes, ⅝ in. in diameter and ½ in. deep. Label the holes in order, one for each of the dilutions.

Before taking a sample, the sirup must be well stirred so that it will be uniform in composition. The size of the sirup sample taken for this analysis is arbitrary; however, the sample must be measured accurately. In this outline the use of a cup for the sample size is suggested because it can be measured with the accuracy required for the test, although any other convenient unit may be used. To conduct the test, the sirup sample is diluted to different volumes with water and the different dilutions tested until one is found which will give a negative test for invert sugar.

Making the dilutions. The test is based upon 5 dilutions by volume of the sirup: 1 + 12, 1 + 20, 1 + 32, 1 + 40, and 1 + 60. These are prepared as described below.

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[4] "Clinitest," manufactured by the Ames Co., Elkhart, Indiana. Mention of commercial items of this kind does not specify recommendation by the United States Department of Agriculture over others on the market.

The 1 + 12 dilution: Measure and pour 2 quarts of water into the mixing pail. Fill the measuring cup exactly to the 1-cup mark with the sirup and pour the measured sirup into the pail. Let the cup drain until the sirup is nearly all removed (sirup drops fall at the rate of one every 30 seconds). Measure a third quart of water and use this to rinse the remaining sirup from the cup. To do this, fill the cup with water, stir, and pour into the pail. Repeat rinsing the cup until the quart of water is completely used. All rinsings are added to the dilute sirup in the pail. The pail should now contain a mixture of one cup of sirup plus 3 quarts of water. Now stir the mixture with the spoon or paddle until it is thoroughly mixed. This gives a 1 + 12 dilution (by volume) of the sirup. Using a clean, dry, 4-ounce tumbler, dip into the dilute sirup and withdraw a half-full tumbler (approximately 2 ounces). This is labeled 1 + 12 and is set aside for later use in making the invert test.

The 1 + 20 dilution: This is made by adding 2 measured quarts of water to the 1 + 12 dilution of sirup already in the pail. After stirring and mixing, using another clean 4-ounce tumbler, remove a 2-ounce sample and label it 1 + 20. Save this for use in the invert test described below.

The 1 + 32 dilution: This is made by adding 3 measured quarts of water to the 1 + 20 dilution of sirup already in the pail. After stirring and mixing, again remove a 2-ounce sample into a clean tumbler, label it 1 + 32, and save for testing.

The 1 + 40 dilution: This is made by adding 2 measured quarts of water to the 1 + 32 dilution of sirup already in the pail. After stirring and mixing, again remove a 2-ounce sample into a clean tumbler, label 1 + 40 and save for testing.

The 1 + 60 dilution: This is made by adding 5 measured quarts of water to the 1 + 40 dilution of sirup already in the pail. After stirring and mixing, again remove a 2-ounce sample into a clean tumbler, label 1 + 60 and save for testing.

Invert Test: Using the samples of diluted sirup prepared above, make the analysis for invert sugar, according to the following directions which are similar to those supplied with the "Clinitest" tablets.

Directions: Place 5 of the test tubes in the test tube block. Fill a clean, dry medicine dropper with the dilute sirup from tumbler labeled 1 + 60. Holding this dropper in an upright position, place 5 drops of this diluted sirup into the test tube located in hole 1 + 60. Using separate clean medicine droppers, in the same manner add 5 drops of each of the remaining 4 samples of diluted sirup to their corresponding test tubes. With a clean medicine dropper add 10 drops of water to each of the 5 test tubes. Remove 5 of the "Clinitest" tablets from bottle onto a clean piece of paper, and then place a tablet into each test tube in order, starting with test tube number 1 + 60. In dissolving, the tablets cause the contents of the tubes to boil. Do not move the test tube while the solution is boiling. Wait 15 seconds after boiling has ceased and then, starting with tube number 1 + 60, fill each test tube, in order, 2/3 full with water. Compare the colors in the test tubes with the first 2 colors of the color scale (which are labeled trace and +) supplied with the "Clinitest" tablets.

INTERPRETATION OF TEST

If the color developed in any of the test tubes is the same or bluer than the first color of the "Clinitest" color scale (trace), it indicates a negative test. If the color is a yellow-green, matching the second color on the "Clinitest" color scale (+), the test is positive. If the color produced is more yellow, or has shades of orange through brown, the test is strongly positive. If the color does not match either of these 2 colors, but is intermediate, the test is considered doubtful, ±. The percentage values given on the "Clinitest" color scale have no relationship to this test for invert sugar in sirup and therefore are to be disregarded.

Examine the tubes in a room illuminated with a tungsten bulb, and with the aid of the first 2 colors of the "Clinitest" color scale record in a

vertical column a plus (+), minus (−), or doubtful (±) for each of the 5 tubes. Then compare this column with those found under the letters in Table 1. Under that column which has markings similar to those obtained in the test are given the minimum, maximum, or probable percentage of invert sugar in the sample tested.

Example 1: If all of the tubes are negative (sample A), then the sirup being tested contained less than 2% invert sugar.

Example 2: If the tube marked 1 + 12 is positive and all the others (1 + 20, 1 + 32, 1 + 40, and 1 + 60) are all negative, sample B, then the test shows that the sirup being tested contained at least 2% but less than 3% invert sugar.

Example 3: If the 1 + 12 dilution is positive, the 1 + 20 dilution is doubtful (positive or negative), but the 1 + 32 dilution is negative, sample C, then the test shows that the sirup contained more than 2% invert sugar but less than 4%, with the probability that the true figure is near 3% or midway between.

Example 4: If, after examining the colors in the 5 test tubes, it is found that the tubes marked 1 + 12, 1 + 20, and 1 + 32 are all positive, and the tubes marked 1 + 40 and 1 + 60 are negative, sample F, this test shows that the sirup being tested contained at least 4% but less than 5% invert sugar.

Example 5: If the 1 + 40 dilution color is doubtful (positive or negative) but the 1 + 60 dilution is negative, sample G, then the test shows that the sirup contained more than 4% invert sugar but less than 6%, with the probability that the true figure is near 5% or midway between these values.

It is quite possible that the sirup being tested has less than 2% of invert sugar and all the dilutions will give negative color tests. In such cases, to determine the color of a positive test it may be desirable to add 3 drops of corn sirup to the 4-ounce tumbler containing the last dilution (1 + 60) and make another color test. Using 5 drops of this solution in a clean test tube, add 10 drops of water and a "Clinitest" tablet. This test will be positive because of the glucose, the reducing sugar contained in the corn sirup.

DISCUSSION

This simple method for the determination of invert sugar is based upon a test which will distinguish between the amounts of invert sugar in the test solution that will react with a "Clinitest" tablet to give either a positive or negative color. For proper functioning the concentrations of invert sugar that will give either a positive or negative test should be within narrow limits. These limits are determined by the colors which readily distinguish between a positive and negative test. The first 2 colors of the "Clinitest" color scale were chosen since they had the greatest difference in hue and were therefore the most easily distinguished. Using these colors, the limits were found to be between 0.10 and 0.15 mg. of invert sugar in the 5 drops taken for the color test. On this basis it was found that dilutions of 1 + 12, 1 + 20, 1 + 32, 1 + 40, and 1 + 60 (on a volume/volume basis) would effectively reduce the invert sugar concentration of sirups containing 1, 2, 3, 4, 5, and 6% invert sugar respectively, so that 5 drops from one of these dilutions will contain more than 0.15 mg., while from a succeeding dilution they will contain less than 0.10 mg. The positive and negative tests at the 5 dilutions for maple sirups containing 1 to 6% invert sugar are shown in Table 1. There will be sirups that will contain an amount of invert sugar which will give a doubtful color test since the

5 drops will contain an amount of invert sugar that is between these limits. In such cases the maximum percentage is indicated by the next larger dilution which gives a negative test.

It was found that the test cannot be applied to sirups containing 7% or more of invert sugar, because there is no combination of dilutions that will give a negative test for one percentage and a positive test for the next higher percentage. The dilution that will give an invert sugar content of less than 0.10 mg. (negative test) per the 5 drops of the test solution for a sirup containing 7% invert sugar will give less than 0.15 mg. of invert sugar (negative to doubtful test) for sirups containing 8 to 9%.

The validity of this simple test was confirmed by analyzing a series of 12 maple sirups whose invert sugar content was established by Schoorl's copper reduction method *(1)*. These sirups contained 1.15 to 6.00% invert sugar. The results obtained are presented in Table 1.

TABLE 1

"Clinitest" color reactions for maple sirups containing different concentrations of invert sugar

Sample No.	A	B	C	D	E	F	G	H	I	J
Dilution (by volume)										
1 + 12	−	+	+	+	+	+	+	+	+	+
1 + 20	−	−	±	+	+	+	+	+	+	+
1 + 32	−	−	−	−	±	+	+	+	+	+
1 + 40	−	−	−	−	−	−	±	+	+	+
1 + 60	−	−	−	−	−	−	−	−	±	+
	Per cent invert sugar by "Clinitest"									
More than	0	2	2	3	3	4	4	5	5	6
Less than	2	3	4	4	5	5	6	6	7	7 (or more)
	Per cent invert sugar by chemical analysis									
	1.15	2.01	2.52	2.96	3.49	3.96	4.67	5.00	5.60	6.00

Legend:
− Indicates a negative color test.
± Indicates a doubtful color test.
+ Indicates a positive color test.

From these results it can be seen that sirups which had an invert sugar content that approached a whole number, yielded 2 adjacent dilutions which gave a positive and negative test that could be easily distinguished and made it possible to determine the percentage within a range of 1% (samples A, B, D, F, H, and J). Sirups whose invert sugar contents lay between whole percentages (samples C, E, G, and I) yielded a positive and negative test with dilutions that were not adjacent, but were separated by an intermediate dilution that gave a doubtful test. Because of this, the percentages could not be determined with assurance closer than ±1%, the limits being marked by the dilutions yielding the positive and the negative test. However, it will be noted that in most cases the percentage of invert was near that indicated by the dilution giving the positive test.

Since these studies were carried out, two other reagents, Sheftel Urine Sugar Test Case[4] and Benedict's Qualitative Solution,[4] manufactured by

Eli Lilly and Company, Indianapolis, Indiana, came to our attention and were subsequently applied to this use. Trial runs have shown that the Sheftel Test was applicable; however, in all cases it gave a negative color reaction one dilution step earlier than the Clinitest. Thus, in order to apply Table 1 to the interpretation of the Sheftel Test, the values for the range of invert sugar must be shifted one column to the left. The Benedict's Solution was found to be a sensitive qualitative test for very low amounts of invert sugar.

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STUDIES ON CANNED CHEESE SPREAD EXPERIMENTALLY INOCULATED WITH SPORES OF *CLOSTRIDIUM BOTULINUM*[a,b]

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Swelling was encountered in some cans of cheese spread containing 5% of smoked meat manufactured commercially for inclusion in Army field rations. A variety of spore-forming bacteria were isolated from the contents of representative swollen cans, but *Clostridium botulinum* was not recovered from any of the cans examined. Portions of the supernatant fluid from the 1 to 5 dilutions of aqueous suspensions of these cheese spread samples were not toxic when injected intraperitoneally into white mice *(7)*.

A study of the microbial flora in canned cheese spread containing 5% of smoked meat was conducted by Huber, Zaborowski, and Rayman *(3)* in the laboratories of the Quartermaster Food and Container Institute for the Armed Forces, Chicago. Isolations of several species of spore-forming bacteria were made from the contents of swollen cans of this product. The isolates were subcultured into differential media. On the basis of the results of these analyses, 18 cultures were selected as representative of the spore-forming organisms in this product. When a composite culture blended from these organisms was inoculated into plain cheese spread, no swelling was observed in cans stored for periods up to 4 months at 90 or 113°F. When a similar spore inoculum was added to culture tubes containing Jensen's spiced meat medium *(4)*, gas formation was observed after 3 to 10 days of incubation at 90°F.

It was realized, however, that toxin production by *Cl. botulinum* was a potential hazard in a vacuum-packed product such as cheese spread. Segalove and Dack *(6)* previously observed in the case of dehydrated meat that growth and toxin production of *Cl. botulinum* occurred when the moisture content was 40% but not 30%. The experiments described in this report were designed to determine whether a spread of the type now used in field rations (minus smoked meat) when stored at 90°F. would be a satisfactory medium for the growth and toxin production of *Cl. botulinum*.

MATERIALS AND METHODS

Approximately four hundred 3-oz. cans of cheese spread were prepared, canned, and stored at the Qm. Food and Container Institute by members of the Institute staff. The spread had the following formula:

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[a] The experiments reported in this investigation were carried out in cooperation with the staff of the Qm. Food and Container Institute for the Armed Forces, Chicago, Ill.

[b] This paper reports research undertaken in cooperation with the Qm. Food and Container Institute for the Armed Forces, and has been assigned number *509* in the series of papers approved for publication. The views or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or endorsement of the Department of Defense.

Ingredients	Weight per batch
Cheddar Cheese[1]	4.7 lbs.
Unsalted butter	4.5 lbs.
$Na_2HPO_4 \cdot 2H_2O$	0.28 lb.
Locust bean gum (sterile)	9.5 g.
Sodium benzoate	4.25 g.
Water[2]	.....
Sodium chloride[3]	.....
Phosphoric acid solution or sodium hydroxide solution[4]	.....

[1] One-half of the samples were made with cheese force-cured for 4 months; the other half with cheese cured 1 month.
[2] Sufficient water to yield a product having approximately 36% moisture.
[3] Sufficicnt NaCl to yield the desired salt level.
[4] Phosphoric acid was used to adjust one-fourth of the samples to near pH 5.2, and sodium hydroxide was used to adjust one-fourth of the samples to near pH 6.2.

The uninoculated spread samples (lots 1-8) were prepared first to avoid contamination with spores of *Cl. botulinum*. A sufficient volume of a phosphate buffer spore suspension containing 7.5 million spores of equal numbers of types A and B *Cl. botulinum* (3 strains of each) was inoculated into the emulsified cheese in the cooker, just prior to the addition of the butter, to give an inoculum of 10,000 spores per g. of cheese spread (lots 9-16).

The following strains of *Cl. botulinum* were used in the inoculum:

Strain	Type	Obtained from
457	A	Dr. L. S. McClung, University of Indiana
62A	A	Dr. K. F. Meyer, Hooper Foundation, University of California
109	A	Dr. K. F. Meyer, Hooper Foundation, University of California
169	B	Dr. K. F. Meyer, Hooper Foundation, University of California
12033	B	National Canners Association
126.7	B	Department of Bacteriology, The University of Chicago

The cheese spread was heat processed in a steam jacketed kettle for 20 minutes. The mixture was heated slowly, with a temperature of 175 to 180°F. being attained just before drawing the batch from the cooker. The spread was immediately transferred to a funnel which fed the previously heated homogenizer. Homogenization was at 1000 p.s.i. (lots 8 and 16 were too viscous to homogenize; hence, these batches were filled into cans from the outlet spout of the cooker). The cans were filled from the discharge pipe of the homogenizer, inverted and sealed as soon as possible after filling. The sealing operation was made on pilot plant sealing equipment. The cans were air cooled while piled one deep on metal trays.

Samples were stored at 90°F. and were subjected to weekly cycling at 40°F. for 16 hours, following which they were returned to the 90°F. incubator. This cycling was done to stimulate practical storage conditions favoring condensation of moisture in portions of the can. Moisture, fat, salt and pH determinations were made by personnel of the Stability Division, Qm. Food and Container Institute, on a representative can of spread from each batch. In addition, pH readings were made on one can from each lot when the contents were sampled for toxin analyses. Results of these analyses are given in Table 1.

The tops of cans were cleansed with alcohol and flamed before opening. Cans from which samples were taken for spore recovery analyses were opened under a hood. The contents of each can were observed for appearance and odor before the samples were taken.

One can from each lot of 5 inoculated samples was selected at random to furnish the spore count inoculum. For the spore recovery analyses decimal aqueous dilutions of the cheese spread were made in sterile 25 × 150 mm. culture tubes. After thorough mixing these samples were heat treated at 185°F. for 10 min. to inactivate any

TABLE 1

Analyses of samples from representative cans of cheese spread stored at 90°F.

Lot No.	Moisture per cent	Fat per cent	Salt per cent	Initial pH	Final pH
1[1]	35.18	.....	1.19	6.1	6.0
2	33.37	.....	2.50	6.0	6.0
3	34.54	47.25	2.48	6.3	6.3
4	34.39	47.71	2.48	5.2	5.3
5[2]	36.21	.....	1.40	6.1	6.0
6	35.42	.....	2.64	6.0	6.0
7	35.99	.....	2.62	6.2	6.1
8	34.98	47.31	2.69	5.3	5.2
9	35.20	48.98	1.29	5.9	5.9
10	35.92	46.87	2.50	5.7	5.8
11	36.53	46.54	2.49	6.1	6.0
12	36.62	45.27	2.55	5.1	5.1
13	36.84	47.28	1.44	5.9	5.7
14	35.85	46.91	2.72	5.9	5.5
15	37.47	45.38	2.64	6.2	6.2
16	35.96	46.01	2.53	5.1	5.1

[1] Lots 1-4 and 9-12 were made with cheese force-cured for 4 months.
[2] Lots 5-8 and 13-16 were made with cheese cured for one month.

vegetative cells present. Duplicate Prickett tubes containing beef infusion agar were inoculated with 1 ml. of the 1 to 10 and 1 to 100 dilutions of the cheese spread. Counts were made after 48 hours of incubation at 86°F.

A Beckman pH meter, Model H2, with glass electrode, was used for the determination of pH on the undiluted cheese spread.

Test mice were injected intraperitoneally with 0.5 ml. of the supernatant fluid from centrifuged 1 to 5 suspensions of cheese spread. The mice were observed for 4 days.

RESULTS

Analyses for toxin production were conducted on 5 cans from each inoculated and 2 cans of each uninoculated lot. Samples were subjected to analysis initially and after 1 and 2 weeks, and 1, 3 and 6 months of storage at 90°F. The 1 to 5 supernatant fluids from all cheese spread samples examined were negative for botulinum toxin.

With the exception of 2 pairs of samples differences in pH readings on the initial and 6-month analyses ranged from 0 to 0.1, although a few greater variations were noted at the intermediate analyses. On samples from lot 13 the difference in pH between the initial and final readings was 0.2 and with lot 14 the difference was 0.4. A slight drop in pH occurred more often than a smaller rise.

All cans examined had a normal appearance and odor.

The spore recovery counts made after 48 hours of incubation at 86°F. are shown in Table 2.

TABLE 2

Spore counts (per gram) on cheese spread cultured in deep beef infusion agar tubes and stored at 90°F. for intervals up to six months.

Lot No.	None	1 week	2 weeks	1 month	3 months	6 months
9	4700	4500	3500	2300	2000	1000
10	5300	4900	4700	3200	3000	1800
11	4100	4500	4000	4400	1600	1900
12	5200	4900	3600	4100	3400	4100
13	3300	4200	4800	2800	1500	500
14	5700	5000	4800	3900	3500	1300
15	4500	3900	3800	3200	1400	300
16	4300	5300	1100	3400	3700	2400

Data in Table 2 showed that approximately one-half of the spores inoculated early in the cooking process were recovered at the initial analyses and that there was a gradual decrease in the spore recovery counts during storage at 90°F. The counts at 6 months of all samples, except lots 12 and 16, were between 7 and 46% of the initial values; lot 12 was 80 and lot 16, 57%.

DISCUSSION

Analyses of 336 cans of cheese spread (5 cans from each inoculated group and 2 cans from each uninoculated group) showed that spreads of the listed composition in Table 1 are not satisfactory mediums for the germination, growth, and toxin production of spores of *Cl. botulinum*. Among the inoculated samples the moisture level ranged from a low of 35.20% to a high of 37.47%. The high percentage of fat added to this product may have contributed to the poor growth qualities of these media even when the brine concentrations (per cent NaCl in H_2O) are low, such as lots 9 and 13 (3.54 and 3.76% brine respectively). The brine percentages in the other inoculated groups ranged from 6.38 to 7.05, which placed them at concentrations that would be expected to be somewhat inhibitory. It is also probable that the emulsifier (Na_2HPO_4) contributed some inhibitory properties to these cheese media. In some of the inoculated samples the pH, moisture content, and salt concentrations are approaching the limits for growth of *Cl. botulinum*. In the other groups two of these three factors are near inhibitory levels. A combination of two or three borderline effects may inhibit growth.

Raising the pH level to near 6.2 in lots 11 and 15 did not cause *Cl. botulinum* to grow. Furthermore, the age of the cheese did not affect the growth of *Cl. botulinum*.

Approximately one-half of the inoculated spores were viable at the initial analysis. These initial counts indicated that all the inoculated spores were not counted when appropriate dilutions were made from a cheese suspension. Several explanations may be offered for the decreased initial counts. Some spores of *Cl. botulinum* may be destroyed by heating the cheese spread at 180°F. prior to canning, and during slow cooling of the cans. Also, heating the diluted cheese at 185°F. for 10 minutes prior to inoculation of the beef agar tubes probably killed additional spores. Furthermore, spores incorporated into an unfavorable medium, such as cheese, may be destroyed at a lower temperature than the same number of spores heated in phosphate buffer. Foster and Wynne *(1)* observed that very small amounts of certain fatty acids would inhibit the germination of spores of *Cl. botulinum*. More recently, Roth and Halvorson *(5)* have shown that oleic, linoleic, and linolenic acids and their methyl esters were inhibitory only when rancid. Their interpretation is that the inhibition is due to the presence of peroxides rather than the unsaturated fatty acids themselves. Soluble starch, which counteracts this inhibition, was incorporated into the beef infusion agar used in making these recovery counts. Even with starch present, there was a possibility that the spore counts may have been lowered by the presence of sufficient inhibitive substances in localized areas of the agar tubes containing 1:10 and 1:100 dilutions of the cheese. In general, there was a gradual decrease in the spore recovery counts during storage at 90°F. Although there was no detectable toxin

formed in any of the cheese samples during storage, some of the spores may have gone into the vegetative state and subsequently died. Halvorson *(2)* observed that some *Cl. botulinum* spores may germinate under conditions unfavorable for growth of the vegetative cells. This may explain the greater loss of viable spores in the more favorable pH range in the cheese stored for 6 months, contrasted to more viable spores where the pH was unfavorable to germination. In both lots 12 and 16 the initial pH readings were 5.1, a pH level usually inhibitory to growth and toxin production of *Cl. botulinum* and these lots had the most viable spores after storage for 6 months. At least part of the lowered counts in the other 6 experimental lots may be attributed to spores germinating but encountering conditions unsuitable for growth.

SUMMARY

Sixteen lots of cheese spread were prepared and stored at 90°F. for intervals up to 6 months. One-half of these samples were prepared from cheese force-cured for 4 months, and the remainder with cheese one month old. These cheese spreads were processed to contain near 36% moisture, to have pH levels varying from approximately 5.2 to 6.2, and salt concentrations of approximately 1.25 or 2.50%.

No botulinum toxin was demonstrated in any of the 8 series of samples inoculated with 10,000 spores per gram of a mixture of types A and B *Cl. botulinum* spores (3 strains of each). Spore recovery counts from the inoculated lots showed that approximately one-half of the inoculated spores were recovered at the initial analysis and that there was a gradual decrease in these counts during storage. All samples contained viable spores at the end of the storage period.

Acknowledgment

The authors wish to acknowledge the technical assistance of Mr. Richard I. Meyer who prepared the cheese spreads used in this study.

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NONENZYMATIC DISCOLORATION OF GREEN BEAN PURÉE*

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The nonenzymatic discoloration of puréed foods (baby foods) has been the subject of many recent investigations. Livingston, Esselen, and Fellers *(5)* found that storage temperature and headspace oxygen were critical factors in the browning of apple sauce. These results were corroborated by Vilece *(8)* who carried out quantitative studies of the effect of oxygen and ascorbic acid on eight fruit and vegetable purées.

It was felt that additional information could be obtained by investigating more extensively the browning of one vegetable product. Green bean purée was selected because it was desired to determine the role of chlorophyll or its degradation products in browning. It was possible to remove all the colored constituents from green bean purée, and thus study browning without the masking effect or reactions of the natural pigments. End products of browning from stored green bean purée were isolated.

EXPERIMENTAL

Green Bean Purée

Preparation of green bean purée. Green bean purée was prepared by washing, trimming, and cutting fresh green beans *(Phascolus vulgaris)* obtained from a local market, steaming for 10 minutes at 121°C. (250°F.), and comminuting in a Fitzpatrick Mill (No. 2 fine mesh screen). The purée was filled into 4½ oz. baby food jars (200 × 309), sealed in a White Vapor Vacuum machine, and processed in a still retort for 45 min. at 116°C. (240°F.). Sample jars were stored at 37.8°C. (100°F.), room temperature (21-26.7°C., 70-80°F.), and 1.7°C. (35°F.).

Isolation of brown compounds from stored samples. Samples of browned green bean purée were taken from an experimental pack that had been in storage for 2½ years. The jars of strained purée exhibited a very dark "top" or oxidative discoloration and a less apparent diffuse "body" discoloration. Samples were taken mostly from the more severely browned top portions of the jars. Each sample of purée was extracted with acetone until no further color was removed. The tissue was then dried under vacuum at room temperature.

Fraction A was isolated from the acetone extract by partitioning the plant pigments into ether, vacuum concentrating the acetone-water solution to a very small volume, and precipitating the brown compounds by pouring the concentrated solution into 10 times its volume of acetone. The cloudy supernatant was decanted, and the brown residue was taken up with a small volume of water and reprecipitated with acetone. The final residue was a water-soluble, brown, amorphous material.

Fraction B was isolated from the brown tissue remaining from the acetone extraction. Considerable brown color was taken up from this tissue by 2 N ammonium hydroxide at room temperature. (Common organic solvents were ineffective.) The ammonium hydroxide was removed by vacuum concentration at room temperature to minimize changes in the brown material due to heat. Purification was carried out by a method similar to the one used for fraction A by pouring the concentrated solution into ten times its volume of acetone, decanting, reprecipitating, and washing with acetone. The final residue was a water soluble, brown, amorphous material similar to fraction A.

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* Contribution No. 968, Massachusetts Agricultural Experiment Station.

Green Bean Pigment

Extraction and measurement of green bean pigment. The prepared samples were analyzed periodically by the following procedure: a 10 g. sample of the purée, from the thoroughly mixed contents of one sample jar stored at each temperature, was extracted with a mixture of 85% acetone and 15% water until all color was removed. The volume of the filtrate was adjusted to 100 ml. by adding acetone or by vacuum concentration. A cloudy solution was clarified by adding a few drops of water. Changes in the pigment fraction of the purée were determined by measuring the absorption spectrum in the visible range (360-700 mμ) with a Beckman DU quartz spectrophotometer.

Identification of chlorophyll degradation products. Chlorophyll degradation products were identified by the Willstätter and Stoll method *(10)* which is founded upon the characteristic basic nature of all magnesium-free chlorophyll derivatives, and their solubility in hydrochloric acid with concentrations up to 32%. Samples from an experimental pack of strained green beans prepared in a commercial pilot plant, as well as samples of laboratory prepared green bean purée were extracted with acetone. Resulting solutions were partitioned with ethyl ether to extract the chlorophyll derivatives. Two ml. portions of the ether solutions were thoroughly shaken once with 2 ml. of hydrochloric acid of the desired concentration. The solubility of the derivatives in the hydrochloric acid as evidenced by color transfer was used for purposes of identification by reference to tables given by Willstätter and Stoll *(10)*.

Chlorophyll derivatives were further identified by chromatographic procedures. Solutions of the pigments from various samples of green bean purée were obtained by complete extraction of the purée with acetone. The pigments were then partitioned into petroleum ether (bp. 30-65°C.) by the addition of water. The petroleum ether solutions were vacuum concentrated to small volumes (approximately 10 ml.), completely dried with sodium sulfate, and chromatographed on columns of powdered sucrose previously dried under vacuum at 60°C. (140°F.) (Zechmeister and Cholnoky, *11)*. A filter aid was unnecessary. Chromatograms were developed with a mixture of petroleum ether and acetone (9 : 1). Pigments were identified by comparison with suitable controls, reference to the literature, and spectrophotometric analysis (Mackinney and Weast, *7;* Willstätter and Stoll, *10;* Gilman, *1)*.

Decolorized Green Bean Tissue

Preparation of decolorized green bean tissue. Green bean purée was prepared by comminuting fresh, uncooked green beans in a Fitzpatrick Mill. A sample of approximately 250 g. of purée was then repeatedly extracted with an acetone-water (9 : 1) mixture and filtered through a Buchner funnel until no additional color was removed. When smaller quantities of decolorized tissue were desired, fresh green beans were comminuted with acetone in a Waring blender and then extracted as above. Either method completely removed the natural pigments, thereby producing a white tissue. Residual acetone was removed from the tissue under vacuum at room temperature. The tissue was analyzed to determine the constituents that remained after the extraction procedure. A Benedict test and a phenylhydrazine test indicated the presence of a very small amount of reducing sugar. Negative ninhydrin tests showed that alpha-amino acids were not present. A weakly positive qualitative Kjeldahl test indicated the presence of a small amount of nitrogenous matter. The bulk of the tissue was cellulose and related fibrous material.

Sealed tube studies. The discoloration of decolorized green bean tissue and the effect of various treatments and added chemicals were studied by the sealed tube method of Vilece *(8)*. The dry tissue was reconstituted to purée consistency by adding distilled water with the appropriate additive (amino acid, sugar, organic acid, ascorbic acid, etc.) in the desired concentration. Ten ml. of purée was then filled into 12 × 125 mm. pyrex test tubes by means of a wide base (7 mm.) pipette. Capillaries were drawn on the tubes, and the headspace oxygen level adjusted to 0.0, 3.3, 20, or 100%. Each test was run in duplicate. The tubes were then sealed, sterilized by processing for 6 minutes at 121°C. (250°F.) in an autoclave, and stored in the dark at 21-26.7°C. (70-80°F.). Controls were frozen and stored at −17.8°C. (0°F.).

Measurement of discoloration. The extent of discoloration of the decolorized tissue after processing and during storage was determined by the method of Livingston and Vilece *(6)* by means of a Photovolt Reflection Meter, Model 610. The reflectance was measured by placing the sealed tubes in a tube holder which exposed a constant area 6×13 mm. to the light source. Reflectance readings were taken at the top and bottom of tubes except where liquid had separated from solid matter. Munsell paper standard 5Y 4/4, which is olive-green in color was set equal to 100% reflectance. A filter was unnecessary. The degree of discoloration was recorded as the "effective surface discoloration" (E.S.D.), which is the difference in reflectance of the surface of a tube before and after storage. Visual observations supplemented reflectance measurements in all cases.

Reaction of decolorized tissue with a glucose-glycine mixture. It was felt that reacting glucose with glycine in the presence of decolorized tissue might give some indication of the possible role of the tissue in Maillard type browning. Twenty-five ml. of a 10% glucose solution and 25 ml. of a 10% glycine solution were refluxed with 1 g. of decolorized tissue for 2½ hours. The resulting reddish-brown mixture was filtered, and the tissue washed thoroughly with water and with acetone until no additional color was removed. Controls were treated with glucose, glycine, and water individually in the same manner. A second test employed acid and alkali digested tissue, and a third, shredded filter paper pulp.

RESULTS AND DISCUSSION

Green Bean Purée

Examination of brown compounds from stored samples of green bean purée. The absorption spectra, in the visible range, of fraction A, obtained from green bean juice, was similar to that obtained by Lewis *(3)* for a fraction of brown material isolated from a glucose-glycine reaction mixture. Both materials showed general absorption which increased sharply at wavelengths approaching the ultraviolet region. The spectral absorption in the ultraviolet region exhibited no maxima or minima, suggesting that no predominant, unsaturated compound was present. Fraction A was an amorphous, brown material and appeared to be similar in general characteristics to substances isolated from apple sauce by Livingston *(4)*, from orange juice by Joslyn and Marsh *(2)* and from apricots by Weast and Mackinney *(9)*. It was felt that further analysis of fraction A, while giving additional information about the chemical nature of the material, would not result in useful information concerning the mechanism of the browning of green bean purée. Furthermore, the concentration of fraction A in browned green bean purée was low as evidenced by the light brown color of the acetone-water solution which remained when the pigments were removed, indicating that the importance of fraction A in the browning of green bean purée was questionable.

Fraction B isolated from green bean tissue was an amorphous, brown material similar in appearance to fraction A. It was water soluble when removed from the tissue. Of the solvents used, only acid or alkali was effective in removing brown compounds from the tissue. Not all the brown compounds were separated from the tissue as the latter remained brown even after the extraction. These two facts show that at least some of the brown compounds present were tightly bound, either physically or chemically, to the tissue.

The visible and ultraviolet absorption spectra of fraction B could not be determined because the solution was of a colloidal nature. Chromato-

graphic adsorption of fraction A or B on activated alumina did not separate the materials into any additional fractions.

Green Bean Pigment

Changes in pigment due to processing and storage. Blanching, processing and storage affected the absorbency of the pigment fraction of green bean purée. (Table 1). Although values varied slightly because of the experimental procedure and sampling, no trend was indicated and

TABLE 1

Absorbency values of pigment extracts from green bean purée stored at ca. 25°C. (77°F.)

Wavelength	Absorbency							
	Fresh	Pureed	Processed	Months storage				
				1	2	3	10	14
mμ								
665	.423	.328	.325	.328	.328	.341	.326	.321
610	.....	.081	.070	.078	.093	.077	.088	.071
535	.....	.104	.096	.110	.123	.108	.117	.104
410	.....	1.08	1.06	1.02	1.04	1.08	1.07	1.05

values may be considered to have remained reasonably constant. The wavelengths recorded represented maxima on the absorption curves; 665 mμ due to the presence of chlorophyll and 535 mμ to pheophytin—a normal acid degradation product of chlorophyll. It was obvious from the results that the conversion of chlorophyll to pheophytin during the blanching process was the major change. The reaction was facilitated by the heating and contact of chlorophyll with natural, acidic plant juices during blanching and comminuting. The pigment fraction did not change significantly during storage.

Chlorophyll degradation products. The solubility of chlorophyll derivatives from green bean purée in hydrochloric acid was determined and compared with data given by Willstätter and Stoll *(10)*. A small amount of color was taken up by 23% HCl, and color was completely transferred from the ether solution to 32% HCl. This indicated the presence of pheophytin and possibly a trace of pheophorbides.

Carotenes are not adsorbed from petroleum ether when chromatographed on sucrose, but merely pass through the column into the eluate. Chlorophyllids, formed from the enzymic degradation of chlorophyll, are strongly adsorbed at the top of a sucrose column and are not resolved with the developing solvent. No chlorophyllids were identified in this experiment. Chlorophylls a (blue-green) and b (green) formed the uppermost bands on the sucrose columns. A series of 3 yellow pigments, probably xanthophylls (Zechmeister and Cholnoky, *11)*, were usually adsorbed next on the column. A broad gray zone identified as a mixture of pheophytins a and b from its characteristic absorption spectra was the lowest band on the column.

Chromatogram 1, Figure 1, shows that with careful extraction procedures the chlorophyll of fresh green beans is not altered. Chromatogram 2 demonstrates that merely comminuting and freezing green beans causes some conversion of chlorophyll to pheophytin. This inversion is probably

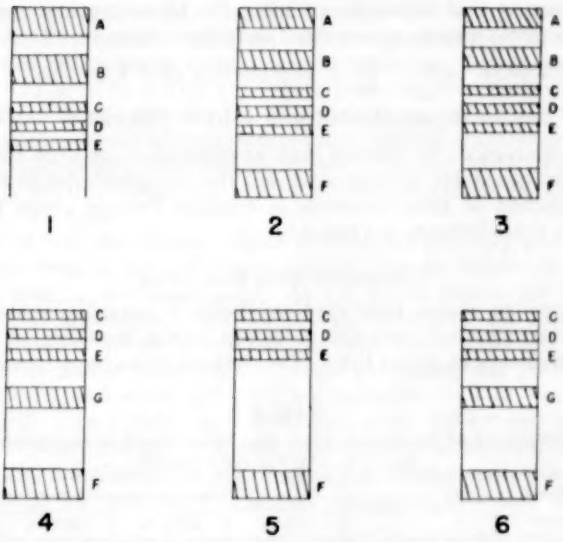

Figure 1. Chromatograms of green bean pigments after various treatments

1. **Fresh green bean purée**
2. **Frozen green bean purée**
3. **Green bean purée processed 5 min. at 240°F. (116°C.)**
4. **Commercial green bean purée**
5. **Fresh green bean purée treated with oxalic acid to convert chlorophyll to pheophytin**
6. **Fresh green bean purée treated with HCl to convert chlorophyll to pheophorbide**

A. Green — chlorophyll b
B. Blue — chlorophyll a
C. Yellow — xanthophyll
D. Yellow — xanthophyll
E. Yellow — xanthophyll
F. Gray — pheophytins a and b
G. Brown — pheophorbides a and b

due to the disruption of the plant cells allowing contact between the natural plant acids and chlorophyll. Chromatograms 3 and 4 show the effects of heat on chlorophyll. A complete breakdown of chlorophyll was found to occur in the commercially prepared sample. Treatment of an acetone extract of fresh green beans with oxalic acid completely converted chlorophyll to pheophytin as illustrated in chromatogram 5. Treatment with HCl theoretically converts the pheophytin to pheophorbide, but this was not found to be a complete conversion. The pheophorbide appeared as a light brown zone above the pheophytin (chromatogram 6). A small amount of pheophorbide was found in the commercial sample as evidenced by the brownish band which developed on chromatogram 4 and by its absorption spectra, which showed a marked similarity to that of pheophorbide. The absorption spectrum of pheophorbide does not exhibit any maxima suitable for spectrophotometric analysis in the presence of chlorophyll or pheophytin.

The reaction that takes place during the blanching and processing of green bean purée may be summarized as follows (Gilman, *1*):

$$\text{chlorophyll (a + b)} \xrightarrow{2H} \text{+ pheophytins (a + b)} + Mg^{++} \xrightarrow{2H} \text{+ pheophorbides (a + b)} + \text{2 phytol}$$

No other chlorophyll derivatives such as chlorophyllids were found. Since pheophytins are stable to weak acid and the pheophorbides to strong acid, further reaction of these products is unlikely because green bean purée remains at a pH between 5.0 and 5.5.

Decolorized Green Bean Tissue

Browning in sealed tube studies. Table 2 partially summarizes the effects of various conditions and chemicals on the browning of decolorized green bean tissues in sealed tubes. Decolorized green bean tissue undergoes

TABLE 2

Discoloration of decolorized green bean tissue stored in sealed tubes at ca. 25°C. (77°F.) for 4 months

Treatment or Additive	Concentration	Effective surface discoloration[1]			
		0% O_2	3.3% O_2	20% O_2	100% O_2
	%				
Stored at 25°C.	.....	7.3	6.5	3.8	0.5
Stored at 1.7°C.	.....	3.5	5.5	0.2	−1.5
Stored at −17.8°C.	.....	3.8	0.8	0.0	−3.0
Control	.....	3.8	4.0	0.3	1.0
Malic acid	0.65	4.5	12.2	9.7	11.3
Citric acid	0.13	2.2	6.5	7.5	6.3
Succinic acid	0.65	3.2	12.3	9.5	6.5
L-leucine	0.5	2.7	6.5	3.5	2.2
L-arginine	0.55	1.7	5.0	1.7	2.3
DL-methionine	0.15	0.5	5.0	5.0	5.0
DL-phenylalanine	0.43	2.8	7.0	4.0	3.0
Glycine	0.5	3.3	7.3	4.8	3.3
DL-glucose	4.0	3.0	7.3	4.7	3.2
D-fructose	4.0	3.8	6.0	3.8	3.3
Sucrose	1.5	2.2	6.5	3.0	3.2
Ascorbic acid	50 mg%	10.3	5.0	−2.7	10.5
Ascorbic acid	100 mg%	−3.2	1.0	7.3	16.5
$NaHSO_2$	1.0	−0.3	−9.2	−2.0	−17.3
$NaHSO_3$	0.06	−7.0	−5.8	−8.3	−3.2
$NaHSO_3$	0.006	−1.7	1.3	−0.5	−4.2
Iron (Fe++)	5 ppm	13.3	8.7	2.3	5.7
Iron (Fe++)	10 ppm	10.3	9.7	2.5	5.4
Pyruvic acid	0.06M	3.8	5.0	−0.5	5.8
Dihydroxy acetone	0.01M	0.6	8.2	0.5	1.2
Ethyl alcohol wash	.....	9.8	9.2	0.5	0.7
Separated pods	.....	1.3	4.3	1.5	1.5
Separated pods + ascorbic acid	100 mg%	1.3	2.5	8.7	18.7
Separated seeds	.....	brown	gray-black	gray-black	gray-black
Separated seeds + ascorbic acid	100 mg%	cream	gray	gray-brown	gray-black

[1] Per cent reflectance after storage subtracted from per cent reflectance before storage indicating the degree of darkening.

a definite storage browning, which is a function of the amount of oxygen in the headspace of sealed tubes and the temperature of storage. Tubes stored at room temperature showed greater browning than those stored at 35°F. (1.7°C.) or 0°F. (–17.8°C.). Ring browning was most pronounced at 3.3% oxygen levels, whereas the browning at higher oxygen levels was more intense throughout the contents, causing general darkening. In some cases the surface of the tubes became lighter at 20 and 100% oxygen levels indicating a further oxidation of brown compounds to lighter colored compounds.

The pH of the reconstituted tissue affected the intensity and the color of the discoloration in thermal processing and storage. Intervals of 0.5 pH units were used in this experiment. At pH 5.5 or higher the darkening assumed a gray-brown color. As the pH was decreased, the color became more orange-brown. At pH 3 or lower, the color was a pinkish-brown. The intensity of the discoloration due to thermal processing was greatest below pH 3, at pH 4.5, and above pH 5.5 when hydrochloric acid was added to adjust the pH. Less discoloration developed at pH 3.0-4.0 and pH 5.0-5.5 (Figure 2). When the pH of decolorized tissue was adjusted to pH 4.0 with disodium phosphate-citric acid buffer, the discoloration was found to be less than in the control even though the citric acid may have been

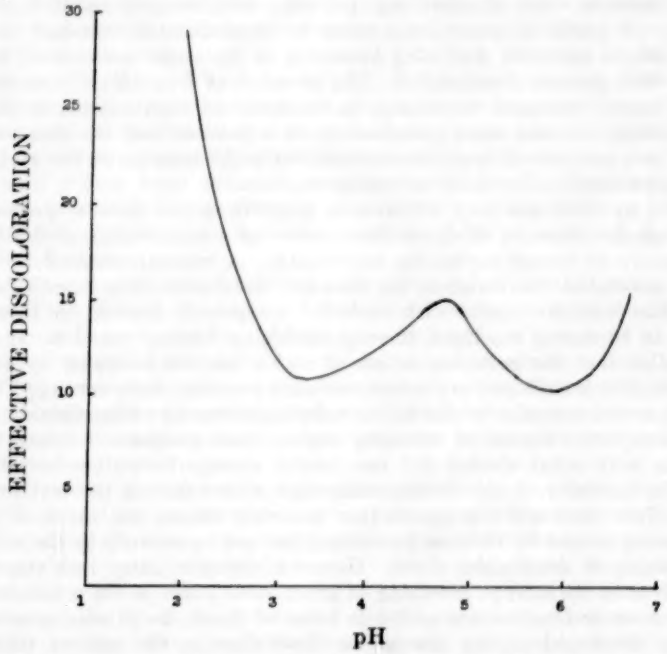

Figure 2. Effect of pH on the discoloration of decolorized green bean tissue in sealed tubes at 20% headspace oxygen content. (Effective Discoloration is the per cent reflectance after processing subtracted from the per cent reflectance before processing.)

directly involved in the browning. When the pH was adjusted to 5.0, the discoloration was found to be greater than in the control, suggesting that citric acid was involved in the browning.

When malic acid and succinic acid were added to decolorized tissue, the degree of browning which developed was considerably greater than that attributable to a lower pH, since the resulting pH value was 3.3 and 3.8, respectively; a range where the pH effect is at a minimum. When citric acid was added to decolorized tissue, the increase in browning may have been due to the lower pH (4.3) or to a reaction of the citric acid itself. Thus, the addition of malic acid and succinic acid increased the discoloration of decolorized green bean tissue, whereas the mechanism of the effect of citric acid was questionable.

At low oxygen levels, ascorbic acid inhibited thermal process and storage browning, but it promoted ring browning at oxygen levels above 9%. As would be expected, 100 mg. per cent was more effective than 50 mg. per cent. When sufficient oxygen was present, the oxidation of ascorbic acid resulted in products which formed brown compounds. This was evidenced by the greater ring browning in the presence of ascorbic acid. At low levels of oxygen the reaction did not proceed as far because of the insufficient oxygen. In the purée of separated green bean pods, ring browning due to the reaction of ascorbic acid oxidation products was very pronounced when compared to controls. One hundred mg. per cent ascorbic acid reduced the darkening of purée of green bean seeds to approximately one-half the intensity of the controls. Any ring browning in the seeds was masked by the very dark process discoloration. The presence of iron (Fe^{++}) in decolorized tissue increased darkening, particularly at high oxygen levels. Ring browning was also more pronounced. It is possible that the iron was involved in a polyphenol type discoloration with the tannins of the seeds, or acted as a catalyst for other browning reactions.

Sodium bisulfite was very effective in preventing the thermal process and storage discoloration of decolorized tissue. A concentration of 0.06% was necessary to reduce darkening appreciably. A concentration of 1.0% not only prevented discoloration, but bleached the tissue. It is conceivable that sodium bisulfite reacts with carbonyl compounds formed as intermediates in browning reactions, thereby inhibiting further reaction. It is also possible that the reducing action of sulfur dioxide liberated by the sodium bisulfite is sufficient to prevent oxidative reactions in browning. The bleaching action may also be due to the reducing action of sulfur dioxide.

The complete removal of reducing sugars from decolorized tissue by extraction with ethyl alcohol did not inhibit storage browning, but did reduce the intensity of the discoloration that occurs during thermal processing. This observation suggests that reducing sugars are involved in the browning caused by thermal processing, but not necessarily in the storage browning of decolorized tissue. However, the possibility that sugars are involved in the storage browning of green bean purée is not precluded. When glucose or fructose was added to tubes of decolorized tissue, greater browning developed during storage in these than in the control tubes. This browning was a function of the headspace oxygen content, i.e., the more oxygen, the greater the browning. Sucrose increased browning slightly, probably through its inversion to glucose and fructose.

Decolorized tissue containing 0.01 M dihydroxyacetone showed a slight increase in storage discoloration when compared with controls. The browning was a function of the oxygen content. This indicates that dihydroxyacetone is a possible intermediate in browning. The effect of dihydroxyacetone was determined because spectrophotometric studies of browned green bean purées indicated the presence of a compound exhibiting spectral absorption characteristics similar to those of dihydroxyacetone. A concentration of 0.067% pyruvic acid caused no increase in storage browning but caused an unexplained decrease in process browning.

Added amino acids increased storage browning slightly in the presence of oxygen. Glycine and phenylalanine caused the most pronounced difference when compared with the controls. This increase in darkening was probably due to a Maillard type reaction of amino acids with reducing compounds present in small amounts in decolorized tissue.

Decolorized green bean pod purée did not undergo so great a process or storage discoloration as the entire tissue. Conversely, decolorized seed purée underwent a very intense gray-black process darkening at oxygen levels above 3.3%. At the latter oxygen level, the discoloration of the seeds appeared as a pinkish-brown color. Added ascorbic acid was effective in reducing the intensity of this discoloration. The browning of the seeds may have been due in part to a polyphenol type reaction because of their high tannin content. Since the seeds contain only trace amounts of reducing sugars, it is unlikely that browning is due to a Maillard type reaction.

In fresh green beans at the time of harvest, the ratio of pods to seeds is approximately 9 to 1 by weight. The intense browning of the seeds, although distributed throughout the entire purée, would contribute considerably to the overall discoloration of the purée. In addition, it is possible that initial compounds or intermediate compounds in browning reactions could diffuse from the seeds throughout the purée. Therefore, it would seem advisable to use young or even slightly immature green beans when preparing purée to minimize the content and size of seeds, thereby reducing discoloration.

The discoloration of green bean juice indicated that some browning occurred through the reaction of its constituents. Most of the brown compounds formed were insoluble and precipitated; although at high oxygen levels, the juice itself had a brown color caused by soluble brown compounds.

It is significant to note that wherever browning occurred in decolorized green bean tissue in sealed tubes, any liquid that separated from the tissue was always colorless, and the browning was intimately associated with the tissue itself.

Reaction of tissue with a glucose-glycine mixture. When a mixture of glucose and glycine is refluxed, a very rapid browning reaction takes place. The initial reaction products are soluble, but when the reaction is continued insoluble brown compounds are also produced. When decolorized green bean tissue was refluxed with a glucose-glycine mixture during the initial stage of the reaction, a portion of the soluble brown compounds remained with the tissue during repeated washing with water and acetone. This condition also occurred when acid and alkali digested tissue was used, but not when filter paper pulp was used. It would thus appear that this reaction is due to the particular nature of green bean tissue. Three explanations

for the reaction are advanced: (a) the brown compounds may be adsorbed on the surface of tissue particles; (b) they may be absorbed into the cellular structure of the tissue; (c) they may react chemically with active groups of the tissue to form brown compound—fiber complexes. The brown tissue obtained by refluxing tissue with glucose and glycine was visually similar to the brown tissue obtained from storage browned green bean purée. The brown material appeared to be tightly bound to the tissue in both instances.

Glucose or glycine, when refluxed with tissue separately, did not react with the tissue. This indicates that intermediate or final reaction products are involved in the browning of tissue when refluxed with a mixture of glucose and glycine.

CONCLUSIONS

No unchanged chlorophyll remains in processed green bean purée. Pheophytin and a small amount of pheophorbide were identified as the only chlorophyll degradation products in green bean purée. These products remained virtually constant during storage, which indicates that they are stable to the conditions in green bean purée. The olive-brown color of the purée is due to the color of pheophytin and pheophorbide. The degradation products of chlorophyll are not involved to any appreciable extent in the nonenzymatic browning of green bean purée.

A procedure for the study of the browning of green bean purée in the absence of natural pigments has been developed. The following conclusions are drawn from the sealed tube study of decolorized green bean tissue:

1. Decolorized tissue undergoes a browning during thermal processing and continues to darken during storage.
2. Storage browning is accelerated by oxygen and by heat (i.e., storage temperature).
3. Maximum browning due to thermal processing occurs above pH 6.0 at pH 4.8, and below pH 3.0. Minimum browning occurs at pH 5.8 and at pH 3.3.
4. Malic, succinic, and possibly citric acid increase storage discoloration more than could be expected from the change in pH.
5. Ascorbic acid inhibits discoloration at low oxygen levels, but causes a marked ring discoloration at high oxygen levels.
6. Iron (Fe^{++}) increases both thermal and storage discoloration.
7. Sodium bisulfite reduces both types of discoloration, probably due to the reducing action of the available sulfur dioxide.
8. Glucose, fructose, and sucrose increase both types of discoloration.
9. The absence of reducing sugars does not inhibit browning caused by thermal processing.
10. Glycine and phenylalanine increase storage discoloration more than leucine, methionine, and arginine.
11. Green bean seed purée undergoes marked discoloration, probably due to a polyphenol type reaction rather than a Maillard type reaction. This discoloration is considered to contribute markedly to the browning of green bean purée during thermal processing.

12. Green bean pod purée and green bean juice discolor moderately during thermal processing and storage.
13. In a model system, glucose, glycine, and decolorized tissue react to form a brown tissue. Similar reactions may take place in green bean purée.
14. Browning of decolorized tissue involves the tissue itself and not the soluble components or free liquid that is present.

It is reasonable to assume that the conclusions listed above apply to green bean purée as well as to decolorized green bean tissue. It was found that the browning of green bean purée is largely associated with the tissue rather than the liquid fraction. Two impure, brown fractions from green bean purée were isolated and described. One fraction was obtained from the liquid portion of the purée; the other from the brown tissue of the purée.

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THE EFFECT OF DIFFERENT METHODS OF COOKING BEEF ROUND OF COMMERCIAL AND PRIME GRADES. I. PALATABILITY AND SHEAR VALUES[a]

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This study was undertaken to learn what method of cooking beef round of Commercial and Prime grades is most satisfactory for palatability and tenderness. Fourteen cooking methods were compared with a standard braising method. These methods were evaluated by means of cooking losses, palatability, and shear.

Cover *(5, 6, 7)* has observed that roasting meat at a very low temperature produces a more tender product than cooking meat in water at the same low temperature or roasting at higher temperatures. She has found that tenderness increases as speed of heat penetration decreases, and has questioned *(6)* whether moist heat is needed for making tough meat tender.

Seasoned and unseasoned meat tenderizers containing papain were tested by Hay, Harrison, and Vail *(8)*. Treated beef was found to be more tender but less juicy than untreated meat. The tenderizer had no significant effect on total cooking losses, aroma, or flavor of the beef. Lowe *(9)* reported that beef treated with a marinade containing vinegar had lower shear values than the control sample.

EXPERIMENTAL PROCEDURE

Paired right and left rounds from 6 steers were obtained from the Chicago stockyards. Four of these animals were from the bottom quarter of the Commercial grade; they were slaughtered under the grading system in use before December 29, 1950. However, the grade of these animals would have been Commercial under the system in use after that date. These 4 animals were of Hereford extraction, from the Middle West, and were about 3½ years old, except the second animal which was about 2 years old. The carcass weights were 572 to 587 pounds. The fifth and sixth pairs of rounds were of average Prime grade, and were purchased after the change in the grading system. The animals from which these rounds were obtained were Hereford steers about 2 years old, from the Middle West. These 2 carcasses weighed 579 and 582 pounds, which is light for Prime.

On the fifth or sixth day after slaughter, the rounds were sliced. Starting at the rump end the slices were 1½, ½, 1½, 1½, ½, 1½, 1½, ½ in. thick. The 1½-in. slices were cooked, while the thinner slices were used raw for chemical assays which are reported in a second paper. Each slice was divided immediately into top round, including the *adductor* and *semimembranosus* muscles, and bottom round, including the *biceps femoris* and *semitendinosus* muscles. The cooking samples of top round weighed 469 to 1439 g.; of bottom round 405 to 1290 g. The samples were stored in a refrigerator until they were cooked 6 to 8 days after slaughter. The cooking methods were arranged

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in groups of 3 or 4, each group including a standard braising method. The methods of one group were distributed among adjacent slices of the right and left rounds to avoid variation from rump to shank end within one group. The groups were alternated between top and bottom muscles in the various animals. Thus, each cooking method was performed on equal numbers of top and bottom muscles during the course of the experiment.

For sampling, the *semimembranosus* muscle of the top round was divided, by means of cuts perpendicular to the outside fat, into 3 parts. The section nearest to the *adductor* and the bone was used for chemical assays (reported in a second paper), the next section for palatability, and the next for shear samples. In the bottom round, the shear samples were taken from the part of the *biceps femoris* nearest the bone, and the palatability samples from the adjacent part of the same muscle. For the chemical samples, the entire *semitendinosus* muscle was used.

Meat was cooked by the following methods:

Standard braising method. A thermometer was suspended so that its bulb touched the bottom of a 10-in. stainless steel frying pan in which 20 g. of the clear fat obtained from heating beef suet was placed. When the temperature reached 392°F. (200°C.), the meat was browned in the fat for 7½ min. on each side. A rack was put under the meat, and a meat thermometer inserted from the side so that its bulb was in the center of the meat. After adding 30 ml. water, a tight-fitting cover was put on the pan and the meat was placed in a gas oven at 300°F. (149°C.). The meat was removed after its temperature had reached 185°F. (85°C.).

Braising to 176°F. and 199°F. These methods were the same as the standard braising method, except that the meat was removed from the oven after its temperature had reached 176°F. (80°C.) or 199°F. (93°C.) instead of 185°F. (85°C.).

Braising without water. The method was the same as the standard braising method, except that no water was added.

Scoring and braising. The meat was scored with a knife, using cuts approximately ¼ in. deep and ¾ in. apart. It was then cooked by the standard braising method.

Pounding and braising. The meat was pounded 70 beats on each side with a metal pounder 1½ in. square and having 12 teeth. It was then cooked by the standard braising method.

Enzymes. A commercial enzyme preparation[c] containing wheat flour, salt, dextrose, and vegetable enzymes derived from Carica papaya was used for all except the first pair of rounds. Following the manufacturer's directions, the meat was sprinkled with 1½ g. of the preparation, and pierced thoroughly on both sides with the tines of a fork. It was then allowed to stand 30 min. at room temperature, and cooked by the standard braising method. The papain content of this product was estimated by measuring its milk-clotting power according to the method of Balls and Hoover *(1)* as modified by Balls and Lineweaver *(2)* except that pasteurized milk was used instead of Klim. Mangel *(10)* has found that the milk-clotting and beef digestion methods give similar estimates of enzyme concentration. The assay of the commercial enzyme preparation and of papain[d] from a chemical supply house indicated their comparative milk-clotting power. Using these data as a guide, a mixture of 0.30 g. papain from a chemical supply house, 0.75 g. flour and 0.45 g. salt was made. This enzyme, which will be called "papain mixture," was used in the same way as the commercial enzyme. No claim is made that these 2 preparations were identical.

Braising with vinegar. The meat was cooked as in the standard braising method except that 15 ml. white distilled vinegar (5% acidity), 15 ml. water, and 24 g. sugar were substituted for 30 ml. water.

Soaking in vinegar. The meat was weighed and placed in a glass dish with 50 ml. white distilled vinegar (5% acidity) and 50 ml. water, and stored 48 hours in a refrigerator. The meat was turned twice during the soaking period. On removal, it was wiped and braised by the standard method except that 30 ml. of the soaking solution and 24 g. sugar were substituted for 30 ml. water.

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[c] M-ten, packed by Petajan Co., Milwaukee, Wisconsin.

[d] Derived from Carica papaya. Supplied by Merck and Co., Inc., St. Louis, Missouri.

Pressure cooking. A 2½-quart pressure saucepan with a 5-10-15 p.s.i.g. indicator, especially equipped with a pressure gauge 2½ in. in diameter and with a thermocouple was used. The thermocouple wire was passed through the cover by means of a rubber and metal bushing, and the metals (copper-constantan for the first pair of rounds; iron-constantan for the others) were fused at the tip. After browning the meat 7½ min. on a side in 20 g. beef fat, a rack was slipped under the meat and 30 ml. water added. The bare thermocouple wire was inserted from the side so that the metal junction was in the center of the meat. The pressure was then raised to 5, 10, or 15 p.s.i.g. and cooking was continued until the internal temperature of the meat was 185°F. (85°C.) as indicated by the thermocouple. Since the thermocouples did not always seem reliable, perhaps because of heat conduction along the wire from the hot steam, the temperature of the meat was checked with a thermometer after removing the cover. If necessary, the meat was cooked under pressure again until it reached the desired temperature.

Roasting at 250°F. and 300°F. The meat was placed on a rack in a frying pan and roasted uncovered in an oven at 250°F. (121°C.) or at 300°F. (149°C.). The meat was removed when its temperature had reached 185°F. (85°C.). Cuts cooked by this method will be referred to as "roasts" in this paper.*

Meat was sliced while hot and served to the judges. The same 7 women served as judges throughout the experiment, an eighth being used for the first 2 pairs of rounds. Aroma, flavor of lean, tenderness, juiciness, flavor of fat, and acceptability were scored using adjectives arranged on a 10-point scale, 10 being the highest and 1 the lowest score.

After the meat had cooled to room temperature, three 1-in. bores were made. These were cut across the grain in a Warner-Bratzler shear. Low shear values indicate tender meat.

Analyses of variance were made for flavor of lean, juiciness, acceptability scores and shear values. In each case, separate analyses were made for each of the 6 groups of cooking methods. Comparisons of methods within each group were made using the error variance for the analysis of this group. Methods in different groups were compared indirectly through the difference between each method and its own standard, using the pooled error variances of the 6 analyses. Animals were also compared using this pooled error term. Values for the 2 enzyme treatments which were missing from the first animal were filled in according to Brownlee *(3)*. The analysis of flavor of lean for the first group of cooking methods is shown as an example:

Source of variation	Degrees of freedom	Mean square	F
Cooking methods (C)	2	0.816	2.45
Muscle (M)	1	0.201	0.23
C × M	2	0.109	0.33
Between animals within muscles	4	0.862	2.59
Remainder	8	0.333	

RESULTS AND DISCUSSION

Cooking data, palatability scores and shear values for animals of Commercial and Prime grades are presented in Tables 1 and 2. Cooking losses were calculated from the change in weight of the meat on cooking, and thus include losses due to both evaporation and drippings. Because of variations in the quality of meat from the rump to the shank end of beef round, the cooking methods are arranged in groups, each group containing a standard braising method. Therefore, direct comparisons should be made within one group rather than between groups. Comparisons of the difference of a method and its standard may be made between groups.

* It is recognized that the slices of beef used are smaller than usual for roasting.

TABLE 1

Cooking data, palatability scores and shear values for beef round of Commercial Grade[1] cooked by several methods[2]

Cooking method	Cooking time per lb.[3]	Total cooking loss	Palatability scores[4]						Shear
			Aroma	Flavor of lean	Tenderness	Juiciness	Flavor of fat	Acceptability	
	min.	%							*lb.*
Braising to 176°F.	34	20.9	7.3	6.8	6.2	6.3	6.2	6.3	26.4
Braising to 185°F. (standard)	34	31.6	7.8	7.2	6.8	6.1	6.4	7.0	22.5
Braising to 199°F.	38	36.5	7.3	6.4	6.0	5.1	5.8	6.0	23.2
Pressure cooking									
5 p.s.i.g.	18	33.2	7.4	6.5	5.7	5.7	6.2	6.0	26.5
10 p.s.i.g.	15	36.4	7.6	6.7	5.6	5.2	6.4	6.0	23.6
15 p.s.i.g.	17	35.6	7.7	7.1	6.0	6.2	6.4	6.6	24.3
Standard braising	33	28.8	7.6	6.8	6.5	6.5	6.5	6.7	27.2
Standard braising	36	32.0	7.4	6.3	6.0	5.2	6.4	6.2	24.8
Roasting at 250° F.	268	36.9	7.6	7.1	7.3	6.0	6.8	6.9	20.4
Roasting at 300° F.	60	28.1	7.4	6.9	6.8	6.7	6.8	6.8	25.4
Braising without water	37	34.0	7.8	7.1	6.6	6.1	6.2	6.8	28.2
Standard braising	38	28.4	8.0	7.3	6.3	6.4	6.4	6.7	25.6
Papain mixture	30	35.9	7.3	6.4	7.8	4.8	6.5	6.8	10.3
Commercial enzyme	33	33.1	7.2	6.6	7.5	5.8	6.1	6.6	18.3
Pounding and braising	31	34.2	7.6	6.8	6.3	5.3	6.2	6.6	20.4
Scoring and braising	33	34.7	7.4	6.6	5.8	5.4	6.5	6.3	23.2
Standard braising	39	30.8	7.5	7.0	6.3	5.9	6.4	6.4	25.0
Braising with vinegar	32	29.4	7.4	7.0	6.1	5.8	5.9	6.3	28.6
Soaking in vinegar	45	32.6	6.2	5.3	6.2	5.5	5.3	5.4	26.6
Standard braising	36	32.0	7.8	7.0	6.7	6.0	6.1	6.6	24.0

[1] Carcass grade was determined prior to Dec. 29, 1950, when the new grading system became effective.
[2] Figures represent averages for 4 animals, except those for the enzyme treatments, which are for 3.
[3] The 15-min. browning time, used for all methods, except roasting, has been included in the cooking time.
[4] Scores are on a 10-point scale, 10 being the highest and 1 the lowest score.

TABLE 2

Cooking data, palatability scores and shear values for beef round of Prime Grade[1] cooked by several methods[2]

Cooking method	Cooking time per lb.[3]	Total cooking loss	Palatability scores[4]						Shear
			Aroma	Flavor of lean	Tenderness	Juiciness	Flavor of fat	Acceptability	
	min.	%							lb.
Braising to 176° F.	30	26.2	8.0	7.5	7.0	6.3	6.5	7.5	18.2
Braising to 185° F. (standard)	42	27.0	7.9	7.9	7.7	7.0	7.6	7.8	16.2
Braising to 199° F.	48	28.4	7.6	7.4	7.4	6.2	7.0	7.3	17.8
Pressure cooking									
5 p.s.i.g.	16	28.8	7.9	7.6	7.6	7.0	7.3	7.7	20.0
10 p.s.i.g.	16	27.8	7.8	7.6	7.0	6.8	6.5	7.5	19.0
15 p.s.i.g.	12	26.6	7.5	7.9	7.4	7.0	7.5	7.8	19.4
Standard braising	37	30.3	7.8	8.2	7.6	7.3	7.6	7.8	24.4
Standard braising	35	25.5	7.1	7.1	7.6	6.4	7.4	7.2	18.0
Roasting at 250° F.	251	31.2	7.8	7.8	8.3	6.5	7.6	7.9	14.9
Roasting at 300° F.	55	24.5	7.2	7.6	8.0	7.0	6.6	7.7	21.2
Braising without water	32	23.4	7.6	7.6	7.2	7.0	7.8	7.5	23.5
Standard braising	30	24.0	7.2	6.7	6.0	5.8	6.8	6.8	20.9
Papain mixture	35	32.7	7.5	6.9	8.4	5.8	7.0	7.0	12.8
Commercial enzyme	32	27.2	7.7	7.5	7.6	6.5	7.4	7.6	16.2
Pounding and braising	28	23.0	8.0	8.0	7.5	7.2	7.4	7.6	18.4
Scoring and braising	33	26.7	7.8	7.8	7.4	6.6	6.8	7.6	22.6
Standard braising	29	23.8	7.4	7.2	7.1	6.2	7.2	7.1	22.1
Braising with vinegar	39	22.8	7.6	7.4	6.2	6.2	6.9	7.0	21.8
Soaking in vinegar	36	24.7	6.7	7.0	6.8	6.3	6.3	6.6	21.8
Standard braising	33	25.8	7.6	7.5	7.0	6.8	7.2	7.4	19.6

[1] Carcass grade was determined after Dec. 29, 1950, when the new grading system became effective.
[2] Figures represent averages for 2 animals.
[3] The 15-min. browning time, used for all methods except roasting, has been included in the cooking time.
[4] Scores are on a 10-point scale, 10 being the highest and 1 the lowest score.

TABLE 3

Significance of differences in scores of three palatability factors and shear values for beef round of Commercial and Prime grades cooked by several methods[1]

Cooking method	Flavor of lean		Juiciness		Acceptability		Shear	
	Mean	Method-Standard	Mean	Method-Standard	Mean	Method-Standard	Mean	Method-Standard
							lb.	
Braising to 176° F.	7.62	−0.43	6.32	−0.06	6.72	−0.56	23.67	3.29
Braising to 185° F. (standard)	7.45		6.38		7.28		20.38	
Braising to 199° F.	6.72	−0.73	5.47	−0.91	6.42	−0.86	21.38	1.00
F	2.45		2.28		2.42		0.91	
Differences 1%								
5%								
Pressure cooking								
5 p.s.i.g.	6.83	−0.45	6.15	−0.62	6.57	−0.46	24.35	− 1.93
10 p.s.i.g.	7.00	−0.28	5.75	−1.02	6.48	−0.55	22.05	− 4.23
15 p.s.i.g.	7.38	0.10	6.45	−0.32	7.03	0.00	22.65	− 3.63
Standard braising	7.28		6.77		7.03		26.28	
F	3.83[2]		5.20[2]		2.17		3.31	
Differences 1%	0.56		0.82					
5%	0.40		0.59					
Standard braising	6.55		5.62		6.57		22.53	
Roasting at 250° F.	7.32	0.77	6.13	0.51	7.22	0.65	18.53	− 4.00
Roasting at 300° F.	7.13	0.58	6.77	1.15	7.07	0.50	23.95	1.42
F	2.37		8.76		1.77		5.17[2]	
Differences 1%			0.92				5.87	
5%			0.64				4.04	

[1] Means are for 6 animals, 4 of Commercial and 2 of Prime Grade. Carcass grade of Commercial animals was determined prior to and of Prime animals after Dec. 29, 1950, when the new grading system became effective.

[2] Indicates significance at 5% level of probability.

TABLE 3 (Concluded)

Cooking method	Flavor of lean		Juiciness		Acceptability		Shear	
	Mean	Method-Standard	Mean	Method-Standard	Mean	Method-Standard	Mean	Method-Standard
							lb.	
Braising without water	7.23	0.15	6.37	0.15	7.07	0.32	26.65	2.65
Standard braising	7.08		6.22		6.75		24.00	
Papain mixture	6.63	−0.45	5.40	−0.82	7.10	0.35	11.85	−12.15
Commercial enzyme	7.12	0.04	6.30	0.08	7.32	0.57	17.78	− 6.22
F	2.10		4.68[2]		1.50		19.19[3]	
Differences 1%			0.94				6.78	
5%			0.66				4.77	
Pounding and braising	7.22	0.20	5.93	−0.07	6.98	0.36	19.72	− 4.28
Scoring and braising	7.02	0.00	5.83	−0.17	6.77	0.15	23.03	− 0.97
Standard braising	7.02		6.00		6.62		24.00	
F	0.18		0.10		0.42		14.02[3]	
Differences 1%							2.85	
5%							1.96	
Braising with vinegar	7.15	−0.02	5.95	−0.33	6.52	−0.33	26.32	3.80
Soaking in vinegar	5.88	−1.29	5.78	−0.50	5.77	−1.08	24.95	2.43
Standard braising	7.17		6.28		6.85		22.52	
F	9.33[3]		2.28		9.88[3]		1.65	
Differences 1%	1.15				0.84			
5%	0.79				0.58			
Comparison of differences between groups								
1% level		1.17		1.24		1.24		7.05
5% level		0.88		0.93		0.93		5.30

[2] Indicates significance at 5% level of probability.
[3] Indicates significance at 1% level of probability.

Flavor of lean, juiciness, acceptability and shear values were selected for analysis of variance. Highly significant correlations were found between flavor of lean and acceptability (+0.81) and between shear and tenderness (−0.55) when all the data were pooled to make 118 paired observations. Results of the analyses of variance are summarized in Tables 3 and 4. Comparisons of cooking methods within each of the 6 groups will be made first. No significant differences were found between meat braised to internal temperatures of 176°, 185°, and 199°F. (80°, 85°, and 93°C.). However, an examination of Tables 1 and 2 shows that meat cooked to 185°F. received slightly higher scores for palatability and had a lower shear value than meat cooked to the other 2 temperatures. Because preliminary tests gave the same indication, 185°F. was selected as the internal temperature for all the other cooking methods.

The judges showed some preference for meat cooked by the standard method over that cooked in the pressure saucepan in spite of the slightly higher shear values for the standard method. Beef cooked by the standard method was significantly (5% level) better in flavor of lean than meat cooked at 5 p.s.i.g. and in juiciness than meat cooked at 5 or 10 p.s.i.g. The analysis also showed that meat cooked at 15 p.s.i.g. compared favorably (5% level) in flavor of lean with that cooked at 5 p.s.i.g. and in juiciness with that cooked at 10 p.s.i.g.

Tables 1 and 2 show that judges' scores for the 2 roasts were generally higher than those for the standard braising method and that scores for the meat roasted at 250°F. (121°C.) usually exceeded those for meat roasted at 300°F. (149°C.), except for juiciness. Indeed, the 300°F. roast was significantly juicier than the standard braised meat (1% level) or the 250°F. roast (5% level). However, the 250°F. roast had a significantly (5% level) lower shear value than the 300°F. roast while the difference between the shear values of the 250°F. roast and the standard braised meat approached

TABLE 4

Significance of differences in scores[1] of three palatability factors and shear values for beef round from six animals

Animal		Flavor of lean	Juiciness	Acceptability	Shear
					lb.
Commercial grade[2]					
Animal No.	1	6.92	5.87	6.68	23.1
	2	6.50	5.72	6.17	31.9
	3	6.80	6.09	6.72	22.8
	4	6.85	5.70	6.40	17.3
Prime grade[2]					
Animal No.	5	7.63	6.85	7.68	17.5
	6	7.37	6.34	7.18	21.3
Differences significant at					
1% level		0.45	0.48	0.48	2.73
5% level		0.34	0.36	0.36	2.04

[1] Means are for 20 cooking methods.
[2] Carcass grades of Commercial animals were determined prior to and of Prime animals after Dec. 29, 1950, when the new grading system became effective.

significance, confirming the work of Cover *(5, 6)*. These results were anticipated by the gross appearance of the meat. Beef roasted at 250°F. appeared dark and hard on the surface, dry and mealy inside, and was so tender it was difficult to cut, while meat cooked at 300°F. looked like an ordinary roast. Cooking losses from the 250°F. roast were not as high as might have been expected from the long cooking time. The results suggest further work on roasting meat at 250°F. using a protective outer covering. It is interesting to note that roasting was just as desirable for beef round of Commercial as of Prime grade.

The enzyme treatments made the meat more tender but less juicy than beef braised by the standard method or without water, a finding which is in agreement with Hay, Harrison, and Vail *(8)*. Shear values were significantly lower for both enzyme treatments than for meat braised by the standard method or without water (1% level except the commercial enzyme and standard differed at 5% level). However, beef cooked with the papain mixture was significantly less juicy than meat braised by the standard method (5% level) or without water (1% level). There was no significant difference in the acceptability of beef cooked by the 4 methods in this group.

Pounding and scoring were without significant effect on the quality of the meat except that shear values were lower (1% level) for pounding than for scoring or braising by the standard method. Pounding and scoring seemed to improve Prime more than Commercial grade meat. Braising or soaking beef with vinegar failed to increase tenderness as measured by judges' scores or by shear values, and lowered scores for flavor of lean and acceptability. The judges scored the flavor of lean significantly (1% level) lower for meat soaked in vinegar than for meat braised in vinegar or by the standard method. The acceptability of meat soaked in vinegar was significantly lower than that of meat cooked by the standard method (1% level) or braised in vinegar (5% level).

In all these comparisons of experimental methods with the standard braising method, it will be noted that in very few cases did the experimental method excel significantly the standard method, indicating that the generally accepted braising method is well suited for cooking beef round. Examination of the data also shows no great differences in the reaction of beef of Commercial and of Prime grades to the cooking methods used.

While not significantly different from the standard, certain methods were different from each other, when the differences of each method from its own standard were compared and tested for significance. Such differences are shown in Table 3. At the bottom of each column headed "method minus standard" are shown the values necessary for significance at the 1% and 5% levels of probability. In such a comparison between groups of methods other than standard braising, the quality of roasted meat is shown to be remarkably good. Indeed, the 250°F. roast was found to excel all other methods (not always significantly) for flavor of lean and acceptability, and it was second only to the 300°F. roast in juiciness. Shear values were significantly lower for the 250°F. roast than for five other methods, while values for the 300°F. roast were not significantly lower than any other method. Making similar comparisons, the 2 enzyme treatments were found to result in lower shear values than the other methods, and to rate fairly

high in acceptability. Pounding before braising excelled significantly several other methods in flavor of lean, acceptability, and tenderness as measured by shear. At the bottom of the list in such a comparison of methods are the 2 vinegar methods and braising to 176° and 199°F., all of which rated low in flavor of lean, acceptability, and tenderness as measured by shear. Meat cooked in the pressure saucepan was low in palatability as compared with that cooked by some other methods; but significantly more tender, as indicated by shear values, than meat cooked by a few of the other methods. These results agree in certain respects with those of Clark and Van Duyne *(4)* who found that the palatability of beef rounds cooked under pressure (15 p.s.i.g.) was lower than that of meat roasted at 300°F. (149°C.).

This experiment offers a valid comparison of the quality of meat from the top and bottom muscles of the round, since each cooking method was performed on equal numbers of top and bottom muscles. However, not one of the 24 analyses of variance made for the flavor of lean, juiciness, acceptability, and shear in each group of cooking methods showed a significant difference between values obtained for the top *(semimembranosus)* or the bottom *(biceps femoris)* muscle used.

A comparison of animals is interesting because of the 2 grades represented. Means for animals have been found by averaging data for all cooking methods, and are shown in Table 4 together with the differences necessary for significance according to the analyses of variance. On the whole, the two Prime animals graded higher than the 4 Commercial animals, although of the 2 Prime animals, 6 was not as good as 5, except in shear values. In flavor of lean the 2 Prime animals excelled the 4 Commercial animals at the 1% level of significance. In juiciness and acceptability, animal 5 was superior at the 1% level to all other animals, including animal 6. However, animal 6 excelled animals 1, 2, and 4 in these 2 factors (1% and 5% levels). Animal 2 was significantly lower (1% and 5% levels) than several other animals of Commercial grade in flavor of lean and acceptability. The shear values were surprising in showing that animal 4 of Commercial and animal 5 of Prime grade were more tender than the remaining 4 animals (1% level). Shear values were higher (1% level) for animal 2 than for animals 1, 3, and 6. Thus, animal 4 was unusually tender for Commercial grade and animal 6 less tender than might be expected for its grade of Prime. An ordinary t-test for the comparison of 2 means indicated that shear values for Prime beef were significantly (1% level) lower than for Commercial beef. A comparison of the 3 palatability factors which were not tested statistically showed that Prime beef received higher scores for tenderness and flavor of fat than Commercial beef, but that aroma scores were about the same for the 2 grades.

SUMMARY

Fourteen cooking methods were compared with a standard braising method using beef round of Commercial and Prime grades, and top and bottom muscles. The effects of these methods on palatability and shear values were studied.

Standard braising was preferred to braising under pressure. The appli-

cation of enzymes before braising made the meat more tender but less juicy than the standard method. Pounding, but not scoring before braising increased the tenderness of beef round. Soaking in vinegar failed to increase the tenderness and lowered the acceptability of the meat. Beef round roasted at 250°F. (121°C.) scored high in flavor of lean and acceptability, and had lower shear values than meat cooked by many other methods. Meat roasted at 300°F. (149°C.) was superior only in juiciness to beef roasted at the lower temperature.

The time-honored braising method may be recommended for cooking beef round. If desired, the meat may be pounded or treated with papain before braising to increase tenderness. Roasting at 250°F. (121°C.) is a superior method for cooking beef round except for the dry appearance of the surface. No significant differences were found in the palatability or shear values of beef from the top *(semimembranosus)* and bottom *(biceps femoris)* muscles of the round. In spite of large differences between animals within a grade, palatability scores were on the whole higher and shear values lower for beef of Prime than of Commercial grade. Little difference was noted in the reaction of beef of the two grades to the cooking methods used.

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THE EFFECT OF DIFFERENT METHODS OF COOKING BEEF ROUND OF COMMERCIAL AND PRIME GRADES. II. COLLAGEN, FAT AND NITROGEN CONTENT[a]

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A comparison has been made of fifteen methods of cooking beef round of Commercial and Prime grades. The cooking methods are described, and palatability and shear values given by Griswold *(5)*. The present paper reports the chemical assays made in this study. The collagen content of the meat, and the moisture, fat, pH, total nitrogen and free amino nitrogen content of the meat and drippings were determined. The content of these constituents, particularly collagen, in raw and cooked meat, meat cooked by different methods and meat of Commercial and Prime grades will be compared.

Although collagen is known to change to gelatin during cooking, little work has been done on the effect of various cooking methods on the collagen content of meat. Bell, Morgan, and Dorman *(2)* found that beef lost 22% of its collagen nitrogen during boiling, 26% during roasting, and observed that losses of collagen increased with longer cooking periods. Smaller losses of collagen on cooking were reported by Hartley and Hall *(7)* for braised beef, 10.95% and 11.6%, depending on the analytical method.

Few observations on the effect of grade on the collagen content of meat have been made. In an early work, Mitchell, Hamilton, and Haines *(10)* observed no correlation between grade of carcass and content of connective tissue in the "ribeye" of beef. Older cattle were found by Mackintosh, Hall and Vail *(9)* to contain more collagen than yearling cattle.

EXPERIMENTAL PROCEDURE

The source of the meat, sampling and cooking methods have been described in detail *(5)*. Paired rounds from 4 steers of Commercial and 2 steers of Prime grade were used. The Commercial beef was slaughtered before, the Prime beef after the grading system was changed on Dec. 29, 1950. Slices of round 1½ in. thick were used for cooking, slices ½ in. thick for chemical assay. Slices were separated into top and bottom round. Cooking methods were divided into groups of 3 or 4, including a standard braising method, and alternating between top and bottom muscles in the various animals. Each group of cooking methods was compared with one raw sample. Chemical samples were from the *semimembranosus* muscle in the top round and the *semitendinosus* in the bottom round. Each chemical sample, including the outside fat, was ground 3 times in a manually operated meat grinder, and frozen. The sample was ground again before thawing. Drippings from the cooked meat were also frozen for analysis.

Moisture was determined by the official method of the Association of Official Agri-

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cultural Chemists *(1)* for drying with heat *in vacuo*. *Fat* was determined by the acid hydrolysis method used by the Quartermaster Corps *(11)*. Samples from the first pair of rounds were digested with acid for 2 hours on a steam bath; samples from the other rounds were allowed to stand with acid for 48 hours at room temperature before digesting 2 hours on a steam bath. Dabkiewicz *(3)* found no difference in the fat content of meat as determined by these 2 digestion procedures. *Hydrogen ion concentration* was determined with glass electrodes on 10-g. samples of meat blended 1. min. with 20 ml. water and allowed to stand 10 min. Replicates of this determination were not done because of the large sample required.

Total nitrogen was found by the Kjeldahl method, modified for the use of boric acid and using meat samples weighing 0.5-0.8 g. The method gave satisfactory recovery of the nitrogen in pure cystine and alanine. *Free amino nitrogen* was determined by formol titration. A blend of 5 g. meat and 45 ml. water was stirred with air which had passed through soda lime, neutralized to phenolphthalein with sodium hydroxide, and 17.5 ml. 35% formalin was added after adjusting to pH 9.2 with sodium hydroxide. The mixture was titrated with sodium hydroxide (approx. 0.1N) to pH 9.2 using glass electrodes. The method of Lowry, Gilligan and Katersky *(8)* was used for *collagen*. Some slight modifications, and a comparison with the Hartley and Hall method *(7)* are given by Griswold and Leffler *(6)*.

The fat content of the *drippings* was determined by chloroform extraction in a separatory funnel. The drippings were heated, then chilled and the aqueous layer drained from the hardened fat layer. The fat was melted, transferred to a separatory funnel with 75 ml. U.S.P. chloroform, and washed with 25 ml. water. The chloroform layer was drawn off and the aqueous layer extracted by 3 successive 50-ml. portions of chloroform. The combined chloroform extracts were evaporated on a steam bath and dried in an oven at 100°C. to constant weight. The aqueous layers were combined, blended, and aliquots taken for moisture, total nitrogen, free amino nitrogen, and pH. Procedures already described for meat were used for these assays.

The significance of losses of collagen on cooking was studied by analysis of variance carried out as already described *(5)*. To compare the means of muscles, the variances for *between animals within muscles* were combined and the pooled error variance subtracted. It was necessary to omit data from the first animal because some connective tissue was left on the blades of the meat grinder, invalidating 9 values. Only 4 of the remaining 5 animals could be used for analysis since an equal number of top and bottom muscles was required. Hence, animal 4 was omitted from the analysis after a random choice between animals 2 and 4.

RESULTS AND DISCUSSION

The analytical data for meat and drippings are shown in Tables 1, 2, and 3. As would be expected, the moisture content of the raw samples was higher, and their fat[c] and protein content lower than that of the cooked samples. Because of the higher fat content of Prime meat, its moisture and protein contents were lower than for the Commercial meat. Differences between the pH of the raw and cooked meat, and of cooked meat and drippings were slight. The pH of the meat and drippings and the moisture content of the meat were considerably higher for one of the Prime samples (animal 5) than for the other animal of this grade.

The total amounts of free amino nitrogen for each cooking method and both grades were calculated, and the amounts in the cooked meat plus

[c] The percentages of fat in raw and cooked samples may not always be comparable because of difficulty in slicing from the round the relatively thin raw sample and the thicker slices for cooking so that the proportions of lean to fat were the same. This may affect the comparison in raw and cooked meat of moisture and nitrogenous constituents.

drippings compared with the amount in the corresponding raw sample. The weights of the meat before and after cooking (Tables 1 and 2) and the weight of drippings (Table 3) were used for this calculation. Under the conditions of this study, the amount of free amino nitrogen in the raw sample exceeded that in the cooked sample plus drippings in 35 of the 40 cases. This fact, together with the fact that a considerably higher percentage of total nitrogen is present as free amino nitrogen in drippings than in cooked or raw meat, suggests that the more soluble nitrogenous constituents go into the drippings, and that the total amount of free amino nitrogen does not increase during cooking. Ginger *et al. (4)* working with broiled steaks also found that a fairly high proportion of the nitrogen in drippings was present as free amino nitrogen. However, these workers found that broiling resulted in the liberation of some free amino nitrogen in contrast to the data reported here on braising and roasting methods. It will be seen in Table 3 that the total amount of nitrogen in the drippings was small. The higher percentages of total nitrogen and of fat in the drippings of the roasted samples were due to the low moisture content of these drippings as compared with those of other cooking methods.

Comparisons of the collagen content of raw and cooked meat, as well as beef of Commercial and Prime grades can be made from the data in Tables 1 and 2. Cooked beef of Commercial grade contained significantly (1% level) more collagen than cooked beef of Prime grade, according to a t-test on the pooled data. Although the average collagen content of raw Commercial beef was higher than that of Prime beef, the difference was not significant, possibly due to the small number of raw samples. The cooked top and bottom muscles did not differ significantly in collagen content, according to a t-test. There was not a significant correlation between the amount of collagen in the cooked meat and either shear values or judges' scores for tenderness (0.032 and –0.140).

Since collagen is known to be hydrolyzed to gelatin on cooking, the finding that raw meat contained more collagen than cooked meat, particularly when moisture content was considered, was expected and gives confidence in the analytical method *(6)*. Collagen losses were found by calculating the total amounts of collagen in the raw and in the cooked meat, using the corresponding weights of raw and cooked meat. A loss of collagen was observed on cooking every sample, the average loss for all cooking methods being 61%. The collagen losses observed were considerably higher than those found by Bell, Morgan, and Dorman *(2)* for boiled or roasted beef or those found by Hartley and Hall *(7)* for braised beef. Results of the analysis of variance of collagen loss are summarized in Table 4. As in the previous paper *(5)*, direct comparisons should be made within one group of cooking methods rather than between groups because of variations in the quality of meat from the rump to the shank end of beef rounds. However, comparisons of the difference of a method and its standard may be made between groups.

Comparisons of cooking methods within each of the six groups will be made first. The data in Table 4 indicate that although losses of collagen on cooking increased as the internal temperature of the meat increased, the differences were not significant. Collagen losses at the two highest pressures

TABLE 1

Analytical data[1] for beef round of Commercial Grade[2] cooked by various methods

Sample	Wt. cooking sample		Moisture	Fat	pH	Total nitrogen	Free amino nitrogen as % total N	Collagen[3]	
	Raw	Cooked						Amount	Loss on cooking
	g.	g.	%	%		%	%	%	%
Braising to 176°F.	710	568	59.2	13.0	5.5-6.2	4.49	8.5	0.659	51
Braising to 185°F. (standard)	889	604	54.6	14.9	5.6-6.4	4.73	7.9	0.673	54
Braising to 199°F.	963	610	54.0	12.3	5.6-6.0	5.17	8.0	0.367	75
Raw			70.4	7.3	5.6-5.8	3.32	12.2	1.130	
Pressure cooking									
5 p.s.i.g.	952	634	56.3	11.9	5.7-6.2	4.89	8.2	0.613	56
10 p.s.i.g.	1047	665	52.2	14.6	5.7-6.1	5.16	8.3	0.313	79
15 p.s.i.g.	1034	668	54.3	13.3	5.6-6.2	5.02	7.8	0.441	71
Standard braising	902	642	51.1	20.2	5.8-6.4	4.45	8.2	0.623	54
Raw			69.8	8.6	5.6-5.9	3.26	11.6	0.916	
Standard braising	822	560	53.0	16.7	5.8-6.1	4.84	8.8	0.500	67
Roasting at 250°F.	776	498	50.4	16.4	5.8-6.1	5.18	9.6	0.462	72
Roasting at 300°F.	825	591	55.9	15.2	5.7-6.2	4.58	8.7	0.548	61
Raw			68.9	9.9	5.6-5.9	3.30	11.1	1.039	
Braising without water	842	562	54.8	15.1	5.7-6.1	4.80	8.1	0.783	38
Standard braising	949	678	53.9	15.8	5.5-6.3	4.58	7.6	0.866	30
Papain mixture	963	618	52.2	18.5	5.6-6.2	4.54	9.0	0.511	61
Commercial enzyme	908	606	55.5	14.2	5.6-6.2	4.75	7.8	0.723	41
Raw			70.7	7.0	5.5-5.9	3.44	12.1	0.872	
Pounding and braising	1033	674	52.4	17.6	5.6-6.2	4.71	8.4	0.497	60
Scoring and braising	946	616	51.8	16.8	5.8-6.2	4.87	8.6	0.445	65
Standard braising	792	549	53.7	15.8	5.7-6.4	4.74	8.9	0.564	50
Raw			67.6	11.0	5.5-6.2	3.27	11.8	0.914	
Braising with vinegar	1013	714	53.2	18.4	5.6-6.1	4.44	9.0	0.545	61
Soaking in vinegar	800	538	56.0	11.5	5.2-5.6	4.87	10.7	0.382	76
Standard braising	805	549	54.5	15.5	5.8-6.2	4.80	9.0	0.604	60
Raw			71.9	6.4	5.6-6.0	3.32	12.0	1.114	

[1] Figures represent averages for 4 animals, except for pH values which are ranges, and for enzyme treatments which are averages for 3 animals.
[2] Carcass grade was determined prior to Dec. 29, 1950, when the new grading system became effective.
[3] Figures for the amount and loss of collagen in the first 9 cooked and raw samples are the averages of 3 pairs of rounds, representing 1 top and 2 bottom muscles.

TABLE 2

Analytical data[1] for beef round of Prime Grade[2] cooked by various methods

Sample	Wt. cooking sample		Moisture	Fat	pH	Total nitrogen	Free amino nitrogen as % total N	Collagen	
	Raw	Cooked						Amount	Loss on cooking
	g.	g.	%	%		%	%	%	%
Braising to 176°F.	873	644	56.2	15.7	5.7-6.7	4.28	8.2	0.493	56
Braising to 185°F. (standard)	786	576	56.8	14.4	5.8-6.8	4.36	8.2	0.316	74
Braising to 199°F.	923	656	52.9	19.0	5.7-6.8	4.41	8.1	0.465	60
Raw			67.2	11.4	5.6-6.3	3.16	10.2	0.836	
Pressure cooking									
5 p.s.i.g.	1026	726	51.1	23.3	5.8-6.9	3.90	8.6	0.367	41
10 p.s.i.g.	1001	720	53.0	21.6	5.7-6.6	3.82	7.7	0.213	68
15 p.s.i.g.	1139	838	49.4	25.8	5.7-6.5	3.75	7.0	0.204	72
Standard braising	924	653	47.8	29.4	5.8-6.8	3.47	8.7	0.350	57
Raw			65.1	15.4	5.6-7.0	2.98	13.2	0.570	
Standard braising	822	612	54.6	19.0	5.8-6.8	4.06	8.1	0.368	82
Roasting at 250°F.	808	556	52.0	20.6	5.9-6.9	4.16	10.7	0.330	85
Roasting at 300°F.	1012	766	55.4	19.0	5.9-6.8	3.96	9.3	0.554	67
Raw			68.6	10.3	5.7-6.8	3.02	13.6	1.448	
Braising without water	810	619	58.0	14.3	5.8-6.7	4.14	8.4	0.706	39
Standard braising	841	638	57.4	14.2	5.7-6.3	4.26	8.2	0.230	80
Papain mixture	924	624	54.5	18.2	5.6-6.6	4.06	8.2	0.255	30
Commercial enzyme	950	698	52.3	22.0	5.7-6.6	3.89	8.2	0.281	76
Raw			66.9	12.0	5.7-6.4	3.20	9.2	0.912	
Pounding and braising	1118	849	54.2	20.8	5.7-6.6	3.84	8.6	0.189	58
Scoring and braising	917	668	51.4	22.2	5.8-6.5	4.08	7.3	0.188	56
Standard braising	861	657	51.4	22.8	5.8-6.5	3.93	9.3	0.187	48
Raw			63.2	18.0	5.6-6.6	2.80	10.7	0.678	
Braising with vinegar	938	728	56.4	16.2	5.7-6.6	4.10	8.8	0.379	62
Soaking in vinegar	1001	756	56.8	15.2	5.6-6.3	4.20	7.8	0.221	80
Standard braising	820	610	54.6	19.4	5.8-6.7	4.00	7.4	0.366	66
Raw			68.8	10.6	5.7-6.6	3.10	11.8	0.856	

[1] Figures represent averages for 2 animals except for pH values which are ranges.

[2] Carcass grade was determined after Dec. 29, 1950, when the new grading system became effective.

TABLE 3

Analytical data for drippings from beef round of Commercial and Prime Grades cooked by various methods

Cooking method	Commercial grade[1]						Prime grade[2]					
	Weight of drippings	Moisture content	Fat content	pH	Total nitrogen	Free amino nitrogen as % total nitrogen	Weight of drippings	Moisture content	Fat content	pH	Total nitrogen	Free amino nitrogen as % total nitrogen
	g.	%	%		%	%	g.	%	%		%	%
Braising to 176°F.	103	74.3	18.6	5.8-6.3	0.77	35.0	152	76.4	16.4	5.8-6.6	0.84	32.7
Braising to 185°F. (standard)	175	75.8	15.3	5.9-6.1	0.88	36.8	124	78.6[3]	14.6	5.8-6.6	0.85[3]	37.1[3]
Braising to 199°F.	244	83.9	9.0	5.8-6.1	0.81	36.4	197	81.8	11.1	5.8-6.6	0.81	33.2
Pressure cooking												
5 p.s.i.g.	211	79.0	13.6	5.8-6.3	0.82	36.3	180	61.6	28.5	5.6-6.5	0.97	33.0
10 p.s.i.g.	229	73.5	17.4	5.2-6.4	0.74	43.6	193	69.6	23.4	5.8-6.5	0.83	31.8
15 p.s.i.g.	245	76.2	15.8	5.8-6.1	0.84	36.8	228	72.0	19.9	5.8-6.5	0.79	31.0
Standard braising	176	77.2	15.9	5.8-6.2	0.76	36.6	160	78.8	14.9	5.8-6.6	0.71	32.2
Standard braising	168	78.1	15.4	5.8-6.3	0.79	36.6	168	80.8	13.0	5.8-6.6	0.68	33.4
Roasting at 250°F.	24	22.9	49.3	5.5-6.2	4.61	37.4	20	12.8	65.4	5.4-6.5	11.20	29.9
Roasting at 300°F.	32	30.6	47.9	5.5-6.0	4.37	33.8	42	30.3	49.0	5.6-6.6	2.28	31.2
Braising without water	138	74.1	17.9	5.9-6.2	0.91	36.9	122	74.9	17.8	5.8-6.4	0.85	33.9
Standard braising	194	83.6	11.0	5.8-6.2	0.77	36.4	134	75.5	17.5	5.8-6.4	0.79	35.2
Papain mixture	234	75.4	18.0	5.7-6.0	0.98	28.8	239	72.7	20.0	5.8-6.4	0.90	26.9
Commercial enzyme	209	82.0	12.3	5.9	0.85	31.8	189	75.2	18.2	5.8-6.4	0.74	31.0
Pounding and braising	265	82.0	11.6	5.5-6.2	0.71	36.1	211	77.2	16.0	5.8-6.6	0.72	32.1
Scoring and braising	224	80.7	12.0	5.8-6.2	0.80	36.0	174	80.5	12.2	5.7-6.5	0.78	33.0
Standard braising	173	77.8	11.9	5.9-6.4	0.84	34.8	145	78.0	15.6	5.8-6.6	0.72	33.2
Braising with vinegar	224	73.6	10.5	5.1-5.3	0.70	44.6	195	74.2	9.4	4.9-5.5	0.61	44.6
Soaking in vinegar	214	75.5	13.2	5.0-5.9	0.80	44.2	204	71.3	12.2	4.9-5.6	0.63	41.5
Standard braising	177	77.4	16.1	5.8-6.2	0.73	37.5	148	79.0	14.6	5.8-6.7	0.74	32.6

[1] Carcass grade was determined prior to Dec. 29, 1950, when the new grading system became effective. Figures represent averages for 4 animals, except for pH values which are ranges, and for enzyme treatments, which are for 3 animals.

[2] Carcass grade was determined after Dec. 29, 1950, when the new grading system became effective. Figures represent averages for 2 animals except for pH values, which are ranges.

[3] Figure is for one sample. The other sample was lost in a laboratory accident.

TABLE 4

Significance of differences in collagen losses for beef round of Commercial and Prime Grades cooked by several methods[1]

Cooking method	Collagen loss	
	Mean	Method-Satndard
	%	
Braising to 176°F.	49.50	−10.25
Braising to 185°F. (standard)	59.75	
Braising to 199°F.	64.00	4.25
F	0.72	
Pressure cooking		
5 p.s.i.g.	51.75	− 7.50
10 p.s.i.g.	71.75	12.50
15 p.s.i.g.	71.50	12.25
Standard braising	59.25	
F	1.10	
Standard braising	74.25	
Roasting at 250°F.	80.75	6.50
Roasting at 300°F.	69.75	− 4.50
F	1.28	
Braising without water	33.25	−27.00
Standard braising	60.25	
Papain mixture	70.25	10.00
Commercial enzyme	55.75	− 4.50
F	5.17[2]	
Differences[4] 1%	36.09	
5%	23.83	
Pounding and braising	62.50	3.25
Scoring and braising	65.25	6.00
Standard braising	59.25	
F	0.95	
Braising with vinegar	63.75	− 2.00
Soaking in vinegar	81.50	15.75
Standard braising	65.75	
F	94.52[3]	
Differences[4] 1%	6.50	
5%	3.93	
Comparison of differences between groups		
1% level		36.90
5% level		27.41

[1] Means are for 4 animals, 2 of Commercial and 2 of Prime grade. Carcass grades of Commercial animals were determined prior to and of Prime animals after Dec. 29, 1950, when the new grading system became effective.
[2] Indicates significance at 5% level of probability.
[3] Indicates significance at 1% level of probability.
[4] Differences are shown only where F is significant.

were greater, but not significantly so, than at 5 p.s.i.g. or for the standard method. The greater collagen loss in the 250°F. (121°C.) roast agrees with the higher tenderness score and lower shear value *(5)* observed on using this method as compared with the values obtained on using the standard method or roasting at 300°F. In the fourth group of cooking methods it

can be seen that meat braised without water lost significantly less collagen than meat cooked by the standard method or treated with the papain mixture (5% and 1% levels). The hypothesis that the amount of water in the meat braised without added water was not sufficient to hydrolyze the usual amount of collagen during cooking is tempting. However, examination of Tables 1 and 2 shows that the moisture content of meat braised without added water was higher than that of meat cooked by many methods which showed higher collagen losses. Although the enzyme treatments had a marked effect on shear values, they did not have a significant effect on collagen losses. This is not surprising since papain would be expected to attack the proteins of muscle rather than of connective tissue. Pounding and scoring had little effect on collagen losses. Soaking in vinegar produced a significantly greater (1% level) loss of collagen than braising with vinegar or by the standard method. Perhaps the greater acidity of beef soaked in vinegar, as shown by a slightly lower pH than for many of the other methods, aided the hydrolysis of collagen.

When the analyses were pooled to compare differences between groups, using the figures in the column headed "method minus standard," the collagen loss of meat braised without water was found to be significantly (1% and 5% levels) lower than that for 8 other methods.

Collagen losses were found to be higher in the top *(semimembranosus)* than in the bottom *(semitendinosus)* muscle of the round for the group of cooking methods using vinegar, but not for any other group of methods nor for the pooled data. A similar lack of significance between values for top and bottom muscles has been noted *(5)* using data for flavor of lean, juiciness, acceptability, and shear. Mean collagen losses for Commercial animals 2 and 3 were 59.4% and 63.8%; those for Prime animals 5 and 6 were 73.5% and 57.2%. The loss for animal 5 was found to exceed those for animals 2 and 6 at the 1% level and that for animal 3 at the 5% level. An ordinary t-test did not show a significant difference between the collagen losses of these Commercial compared with the Prime animals.

SUMMARY

The collagen content of meat, and moisture, fat, pH, total nitrogen and free amino nitrogen of meat and drippings have been determined for beef round of Commercial and Prime grades cooked by 14 experimental methods and a standard braising method. It was found that the more soluble nitrogenous constituents went into the drippings, but that the total amount of free amino nitrogen did not increase during cooking. The collagen content of cooked beef was significantly higher for Commercial than for Prime grade, but was not significantly different for the top and bottom muscles of the round. The average collagen loss on cooking by all methods was 61%. Losses of collagen on cooking increased as the internal temperature of the meat increased, and were greater when the meat was cooked at 10 or 15 p.s.i.g. than at 5 p.s.i.g. or by the standard method. Collagen losses were higher in meat roasted at 250°F. (120°C.) than at 300°F. (149°C.) or braised by the standard method, and were especially high in meat soaked in vinegar. Collagen losses in meat braised without added water were lower than for any other method. Treatment of the meat

before cooking by pounding, scoring or the use of enzymes had little effect on the loss of collagen. There was not a significant difference in the collagen losses from top and bottom muscles of the round for the pooled data.

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NUTRITIONAL ADEQUACY OF A SEMI-SYNTHETIC DIET STERILIZED BY STEAM OR BY CATHODE RAYS

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Exploration of different ways to sterilize foods for use in germ-free life studies led to this study of growth and reproduction in mice fed a semi-synthetic diet which was (a) non-sterile, (b) steam sterilized, and (c) cathode ray sterilized. The work reported herein gives evidence of the nutritional adequacy of the sterilized diets for three generations of mice.

Proctor and Goldblith *(3)* have reviewed the chemical reactions which occur during the sterilization of foods and selected nutrients by different radiations. Little information is available about the biological adequacy or toxicity of diets sterilized by radiations with high voltage electron beams. The lack of such information appears to be a deterrent to the use of such techniques, at least in Britain *(1)*. Proctor *(2)* has fed rats for one year with a cathode ray treated diet. The work of the group at the Michigan University Engineering Research Institute[b] shows that no acute toxicity is elicited by treatment of diets with gamma rays.

EXPERIMENTAL METHODS

Two pregnant mice of the Swiss strain were selected from the stock colony and fed the cathode ray sterilized diet L-109 through parturition and lactation. At weaning the littermates were divided into two groups: one half were fed the non-sterile diet and the other half were fed the cathode ray sterilized diet. The performance of these offspring is recorded as the first generation. Only mice from the first 2 litters weaned on each diet (4 to 7 females and 2 to 3 males) were kept for breeding. Their young comprise the 2nd generation. The experiment was terminated when the litters born to the 3rd generation were weaned. The work with mice fed the steam sterilized diet was completed through 4 generations before the other groups were started. The data does allow a rough comparison of results obtained with one diet sterilized by two different methods.

The mice were maintained in jars with deep wood shaving litter. The sexes were unfortunately not identified when the mice were weighed. Pregnant mice were isolated for parturition and lactation. Food and non-sterile tap water were supplied *ad libitum*.

The non-sterile and cathode-ray sterilized diets were at all times refrigerated until presented to the mice. The steam sterilized diet was kept at room temperature until fed. The composition of the high vitamin, semi-synthetic diet used is given in Table 1. The ingredients were prepared in powder form and mixed to homogeneity. The non-sterile diet was made as 2 batches and stored throughout the experiment. The diet to

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[b] Drs. H. C. Eckstein, B. W. Uhlendorf and L. E. Brownell (Progress Report No. 5) have reported tests with rats fed cobalt 60 ray treated milk and Drs. H. C. Eckstein, H. O. France and L. E. Brownell (Progress Report No. 6) have reported preliminary work with rats fed a solid diet sterilized with cobalt 60.

TABLE 1

Solid diet L-109

Constituent	Grams per 100 g.
Casein (Labco)	25
Starch (corn)	54
Cellophane	2
Oil (corn)	6.8
Minerals (Salts L-11)	6

Salts L-II

	grams
$CaCO_3$	1.80
$CaHPO_4$	0.33
K_2HPO_4	1.35
Na_2HPO_4	1.20
NaCl	0.30
KI	0.0005
$MgSO_4 \cdot 7H_2O$	0.45
$MnSO_4 \cdot 4H_2O$	0.075
Fe citrate (ie)	0.45
$CuSO_4 \cdot 5H_2O$	0.023
$CoCl_2 \cdot 6H_2O$	0.003
$ZnSO_4 \cdot 7H_2O$	0.006
$Na_2B_4O_7 \cdot 1H_2O$	0.003
$AlK(SO_4)_2 \cdot 12H_2O$	0.005

Yeast and liver extracts	5 g.
Vitamin A esters	800 IU
Ascorbic acid	200 mg.
Vitamin D_3	100 IU
α-tocopherol (Vit. E)	50 mg.
Mendadione (Vit. K)	10 mg.
Thiamin Cl	6 mg.
Riboflavin	3 mg.
Nicotinamide	5 mg.
Ca pantothenate	30 mg.
Choline Cl	200 mg.
Pyridoxine Cl	2 mg.
Pyridoxamine Cl	0.4 mg.
Biotin	0.1 mg.
Inositol	100 mg.
Pteroyl glutamic acid	1 mg.
p-aminobenzoic acid	5 mg.

be sterilized with cathode rays was packaged 0.6 g./cm.^{3} in double polyethylene bags, 10 cm. wide and about 1 meter long. It was prepared in one batch at the University of Notre Dame (June 9, 1953), shipped to the High Voltage Engineering Corporation, at Cambridge, Mass., treated by one passage under a scanned electron beam a few inches from the aluminum window of a 2 MeV Van de Graaf accelerator with a minimum dosage of 2×10^6 rep (average was 2.8×10^6 rep), and shipped back on June 25, 1953. The conveyor belt upon which the diet traveled moved at a speed of one meter per minute. Beam current was 250 microamperes and the scan width was 15 cm. The diet to be steam sterilized was placed in cloth bags in a layer one inch thick, placed in an autoclave with the jacket hot and subjected to a 5-10" vacuum for 5 minutes; 17 p.s.i. steam was then admitted to the diet in the chamber to give a temperature of (121°C.) for 25 minutes and a vacuum was drawn for about 40 minutes while the diet cooled to 50-60°C. This diet was made at intervals of 2-3 weeks.

Both sterilization procedures were shown to give bacteriologically sterile diets. Sterility tests were run on the cathode ray treated diet as follows: Diets seeded with indicator test organisms were prepared by mixing 1 ml. aqueous *Bacillus stearothermophilus* spore suspension (3×10^6 viable spores/ml) per 100 g. of diet. The number of *Bacillus globigii* spores used as inoculum was unknown. Seeded diets were checked for sterility by inoculating 15 samples from each of 4 segments of the 1 meter long diet bags into deep tubes of fluid thioglycollate medium. Five tubes from each segment were incubated at 55°C., 37°C. and room temperature (ca. 25°C.). Similar techniques were run using starch-tryptone-glucose-extract broth at 55°C. as a spore germination medium for *B. stearothermophilus* and at 37°C. for *B. globigii*.

All unseeded diets (containing only the indigenous organisms) and the *B. globigii* seeded diets tested sterile after cathode ray treatment. Two of the 3 *B. stearothermophilus* seeded diets tested sterile under similar treatment. The survival of *B. stearothermophilus* spores occurred in the first of the 3 samples irradiated and may possibly have been due to unequal thickness of the diet layer during irradiation. Close observance of dosimetry and diet layer thickness resulted in sterilization in the next 2 samples. It may be mentioned that further evidence of the sterility affected is provided by routinely feeding germfree chicks and poults small quantities of cathode ray treated antibiotics in starch or lactose filler over a period of 3 years.

RESULTS

Analyses to determine the destruction of certain nutrients, summarized in Table 2, were performed upon a single batch of diet. The analytical methods have been reported *(4)*. The nitrogen values indicate the diet did not increase in moisture content from the

TABLE 2

Vitamin loss during sterilization of Diet L-109[1]

	Fresh	Sterilized	
		Steam	Cathode Ray
Nitrogen, %	3.55	3.48	
Thiamin, γ/g.	69.4	14.0	20.0
Riboflavin, γ/g.	29.0	26.7	32.7
Niacin, γ/g.	110	107	127
Pantothen, γ/g.	324	224	315
Folic Acid, γ/g.	5.98	4.41	6.22

[1] Each value is the average from analyses of duplicate samples of one or more batches of diet. The nitrogen determination was made when no yeast or liver was in the diet. The vitamin analyses were run on a water extract of the diet since this procedure gave higher values generally than the analyses on enzyme digested diet.

steam treatment. The large quantities of vitamins incorporated in the diet assured an adequate quantity of each despite losses during sterilization. Steam sterilization produced greater vitamin losses than did cathode ray sterilization.

The growth data from 3 generations of mice, summarized in Figure 1, give preliminary evidence that mice fed the cathode ray sterilized diet grow at about the same rate as do mice fed the un-treated diet. Mice fed the steam sterilized diet may grow at a somewhat faster rate than do mice fed either the un-treated or the cathode ray treated diets; however, the low numbers of mice involved do not give significance to this observation.

The reproduction data (Table 3) show that all females fed the un-treated and cathode ray sterilized diets reproduced while only 80% of the mice fed the steam sterilized diets reproduced during the first four months of life. Mice fed non-treated, cathode ray sterilized or steam sterilized diets cast litters at similar ages (the average 3 generations were 78, 78 and 75 days, respectively) and the average number of mice weaned per litter were very similar (the average being 6, 5 and 5 mice weaned, respectively. The percent of young weaned was lower for mice fed treated diets. The average size of the young at weaning (21 days) was 9.9 for mice fed the un-treated

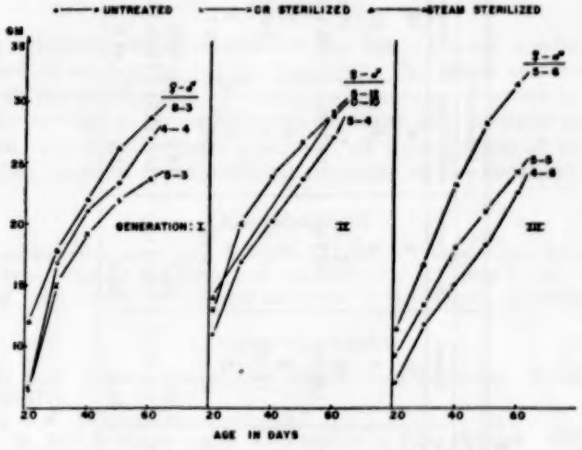

Figure 1. Growth record of mice fed Diet L-109.

diet, 11.2 g. for mice fed the cathode ray sterilized diet and 13.2 g. for mice fed the steam sterilized diet. When the average values for the number and the size of the young at weaning are multiplied together, the product is the total weight of young produced by the dam during pregnancy and lactation (disregarding any solid food eaten prior to 21 days). Assuming a relatively constant weight for the total litter at birth and a positive correlation between the amounts of milk and solid food consumed by the young mice, this product may be considered to be a rough index of lactation. The average lactation index was 68 for the 13 dams fed the non-treated diet, 58 for the 17 dams fed the cathode ray sterilized diet, and 66 for the 25 dams which produced young when fed the steam sterilized diet.

The gross appearance and activity of all mice was good throughout the experiment. No differences were noted in mice fed the 3 diets excepting one of the third generation dams fed the cathode ray sterilized diet developed a large tumor during the course of lactation. She weaned this litter despite her hardship. This was the only tumor noted.

DISCUSSION

The results indicate that sterilization of a high vitamin, semi-synthetic diet with cathode rays or steam does not produce any consistently harmful effects when it is fed to 3 full generations of mice. Since mature stock (mice) were fed the cathode ray sterilized diet during parturition and the full course of lactation, and since the 4th generation mice nibbled some diet during their third week of life, the cathode ray sterilized food was eaten by members of 5 successive generations of mice during the period of July 14, 1953 to April 16, 1954. It would appear that cathode ray sterilization of such a diet does not produce any serious toxic effects.

It should be emphasized that the diet was designed to provide adequate nutrition even if 98% of some of the vitamins were destroyed. Thus the destruction of ⅔ of the thiamin during sterilization of the diet did not lead to a thiamin deficiency. None of the other vitamins tested showed significant loss. Different results might have been anticipated had this diet contained water. In this respect the diet used resembled foods like dried milk or flour more than fresh milk or meat.

TABLE 3

Reproduction of mice fed Diet L-109

	1st Generation			2nd Generation			3rd Generation		
	Non-treated	Cathode Ray	Steam	Non-treated	Cathode Ray	Steam	Non-treated	Cathode Ray	Steam
No. Dams	4	6	6	5	7	10	4	4	14
No. with litters[1]	4	6	6	5	7	6	4	4	13
Age at 1st litter	71	76	62	91	70	73	71	88	90
(Range)	(68-75)	(82-101)	(60-63)	(68-113)	(53-111)	(68-83)	(69-74)	(69-105)	(63-130)
Litter size, Ave.[1]	5	6	6	9	9	8	8	9	7
(Range)	(3-6)	(3-11)	(3-10)	(8-9)	(6-11)	(6-10)	(6-10)	(7-10)	(5-9)
No. weaned, Ave.	4	4	4	8	7	5	8	5	6
(Range)	(0-6)	(0-6)	(3-6)	(7-9)	(0-11)	(3-6)	(6-10)	(0-8)	(5-7)
Wgt. at weaning, Ave.	11.1	(12.0-14.0)	(12.9-15.1)	7.2	10.5	12.0	11.3	10.2	13.5
(Range)[2]	(10.2-11.6)	13.0	14.0	(5.6-10.5)	(7.7-14.4)	(8.0-13.3)	(8.7-14.3)	(10.0-11.5)	(13.0-14.0)
Lactation Index	44.4	52.0	56.0	57.6	73.5	60.0	90.4	51.0	81.0

[1] Some of litter may have been eaten before they were recorded.
[2] Litter averages.

SUMMARY

A high vitamin, semi-synthetic diet was fed to limited numbers of three generations of mice after (a) no treatment, (b) steam sterilization, and (c) cathode ray sterilization. Vitamin losses were more severe in the steam sterilized diet than in the cathode ray sterilized diet. General appearance, growth and reproduction were similar in all three groups of mice to give biological evidence of the nutritional adequacy of the sterilized diets.

Acknowledgments

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THE ACTION OF TOMATO AND AVOCADO POLYGALACTURONASE*

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The action of tomato and avocado enzyme preparations on polygalacturonic acids was compared with that of fungal polygalacturonase (PG). Fungal, tomato, and avocado preparations hydrolyzed polygalacturonic acid and also tri- and digalacturonic acid to galacturonic acids. The preparations also hydrolyzed methyl digalacturonic acid to galacturonic and methyl galacturonic acid. Their specificities seem to be qualitatively the same and their apparent differences lie mainly in their relative rates of hydrolysis of oligouronic acids, such as digalacturonic as compared with polygalacturonic acid. The rate of hydrolysis of digalacturonic acid is much lower with the fruit enzymes than with a preparation of fungal origin.

Enzymes that cleave the glycosidic linkages of pectin seem to consist of at least three types. Polymethylgalacturonase (PMG) hydrolyzes esterified pectic substances to materials of low molecular weight without production of detectable amounts of monomer. Such enzymes have been described by Seegmiller and Jansen *(21)*, Roboz *et al. (18)*, and Ayers *et al. (1)*. Polygalacturonase (PG), the most widely studied pectin-glycoside-splitting enzyme, degrades de-esterified polygalacturonic acid ultimately to galacturonic acid *(7)*. A third type of enzyme hydrolyzes de-esterified pectic substances to oligogalacturonides and galacturonic acid. Demain and Phaff *(4)* and Demain *(3)* described the action of a yeast polygalacturonase (YPG) and showed that ultimately galacturonic and digalacturonic acids were produced. This enzyme produced galacturonic acid from tetra- or trigalacturonic acid but failed to hydrolyze digalacturonic acid.

The pectin-glycoside-splitting enzyme of tomatoes *(9, 10, 11, 17)* was named depolymerase (DP) by McColloch and Kertesz. This enzyme was reported by McColloch and Kertesz to act in a manner different from polygalacturonase. Its action on pectic acid ceased at about 50% hydrolysis with the production ultimately of digalacturonic acid, and no galacturonic acid was found among the hydrolysis products. Paper chromatography was not then available to McColloch and Kertesz for the study of the action of this enzyme and their conclusions regarding the limits and end products of hydrolysis were based upon analysis of reducing groups and application of the Ehrlich lead acetate test for galacturonic acid *(6)*.

McCready and McComb *(15)*, Roelofsen *(19)*, and Luh *et al. (8)* presented preliminary results showing that galacturonic, di-, tri-, and a series of oligogalacturonic acids are produced by a tomato enzyme acting on pectic substances. We have now conducted further studies to determine the

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nature of the action of the tomato enzyme. Its action was compared with that of fungal and avocado preparations on polygalacturonic, tri-, and digalacturonic acids. The tomato and avocado enzymes hydrolyze polygalacturonic acids ultimately to galacturonic acid, convert digalacturonic acid to galacturonic acid, and methyl digalacturonic acid to methyl galacturonic acid and galacturonic acid.

It appears that these three enzyme preparations are qualitatively similar and belong to the class of polygalacturonases. There seems to be no justification for postulating the presence of more than one pectin-glycoside-splitting enzyme in these preparations and references to the tomato enzyme solely as "depolymerase" seem to be undesirable in the light of the similarity of its action to that of fungal polygalacturonase (PG). It appears that the tomato enzyme shows a size specificity quantitatively different from that of fungal polygalacturonase. The rate of hydrolysis of digalacturonic acid, compared to the rate of hydrolysis of polygalacturonic acid, is less for the tomato than for the fungal enzyme.

METHODS AND MATERIALS

Polygalacturonic acid was prepared by boiling a commercial citrus pectin (Pectinum N.F. VII, manufactured by Sunkist Growers, Ontario, California)[b] in 1*N* sulfuric acid for 1 hour, and then isolating and purifying the product as previously described *(13)*. Its molecular weight was 3400 as determined by reducing group analysis *(7)*, and its anhydrouronic acid content determined by a colorimetric method *(12)* was 99%.

Commercial sodium pectate (Sodium Polypectate, manufactured by the Sunkist Growers, Ontario, California),[b] without further purification, was used as a substrate for some of the enzyme actions. This substance is prepared commercially by enzymic de-esterification of orange pectin *in situ (2)*, extracted with one of the polyphosphates, and isolated by solvent precipitation. The product is a mixture composed chiefly of sodium pectate of high molecular weight and containing substantial amounts of araban and galactan. Its anhydrouronic acid content was 65% (moisture-free basis).

Crystalline dibrucine digalacturonic acid [dibrucine (4-[α-D-galactopyranosidurono]-D-galactopyranosid) uronate] and crystalline tribrucine trigalacturonic acid were prepared as previously described *(13)*. Before use as substrates for enzyme actions the brucine was first removed by treatment of the solutions with Dowex 50 cation exchange resin and the pH was then adjusted to 4.0. Crystalline methyl digalacturonic acid [methyl (4-[α-D-galactopyranosidurono]-D-galactopyranouronic acid)] was made from digalacturonic acid by refluxing it with dry hydrochloric acid in methanol followed by hydrolyzing the ester groups with alkali as previously described *(16)*.

Pectinol 100D (manufactured by Rohm and Haas Co.)[b] and purified polygalacturonase (PG) made from Pectinol 100D by the method of Jansen and MacDonnell *(7)* were used in these experiments. Tomato enzyme was made essentially as follows: Four hundred grams of improved Pearson variety, red-ripe tomatoes were blended with 400 g. of 5% sodium chloride solution in an electric blender. The pH was adjusted to 6.5 with 1*N* sodium hydroxide solution and blending was continued for 3 minutes. The tomato pulp was screened out and the remaining insoluble solids removed by filtration on diatomaceous silica filter aid. The clear extract was made to 0.9 saturated with ammonium sulfate and the precipitated protein was collected by centrifugation. The precipitate was dissolved in 40 ml. of 1% sodium chloride solution and, after thorough stirring, the undissolved material was removed by centrifugation. The solution containing the enzyme was dialized overnight in a cellophane sausage casing against distilled water. One per cent sodium chloride solution was added and the solution was preserved at 0°C. in the presence of phenylmercuric nitrate.

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[b] Mention of manufacturers or trade names does not imply that they are endorsed or recommended by the U. S. Department of Agriculture over others of a similar nature not mentioned.

Avocado enzyme from soft ripe fruit was prepared essentially in the manner as previously described for tomato, except that seeds and skins were removed before blending. The strained juice, after blending with salt solution, was filtered and the clear extract treated as described for the tomato enzyme preparation.

The reaction course of the tomato enzyme on polygalacturonic acid at pH 5.0 and 25°C., as compared with fungal polygalacturonase, was followed quantitatively by a hypoiodite method *(7)* for determining the increase in reducing groups. The results are shown in Figure 1.

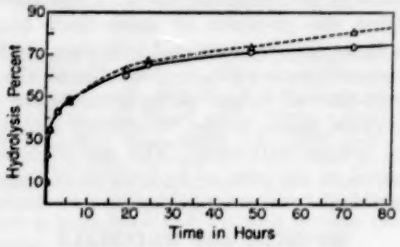

Figure 1. Production of reducing groups vs. time during hydrolysis of polygalacturonic acid by tomato enzyme (circles) and fungal polygalacturonase (triangles).

An amount of the tomato enzyme (1 ml. of concentrate per 25 ml. of digest) was used so that its initial rate of hydrolysis of polygalacturonic acid was the same as for 0.0015 g. or 0.03 (PGu) of the fungal preparation. After about 10 hours 50% of the total uronide bonds had been hydrolyzed and the rate of hydrolysis dropped rapidly. From 50% on, these enzymes were hydrolyzing such substrates as di-, tri-, and tetragalacturonides. Although the rate of hydrolysis is slow, after 72 hours the tomato enzyme was still producing galacturonic acid at the expense of one or more of the oligogalacturonides. The rate of hydrolysis of one or more of these substances was slower in the case of the tomato than that of fungal enzyme.

The reaction courses of the tomato and fungal enzyme preparations were followed qualitatively by paper chromatography. Ascending irrigation for 16-20 hours on Whatman No. 1 filter paper with a miscible solvent composed of 5 vol. of ethyl acetate, 3 of water, and 2.5 of acetic acid was used as previously described *(13)*. After irrigation the paper was dried for 2 hours without heating. The sheet was then dipped into an ethyl acetate solution containing 2% each of aniline and trichloroacetic acid. After drying, the sheets were heated 5 minutes at 85°C. to reveal the positions occupied by the reducing substances. Galacturonic, di-, and trigalacturonic acid were run on the same paper sheets and tentative identification of the unknown spots was made by comparison with these standards.

Fungal, tomato, and avocado polygalacturonase preparations hydrolyzed polygalacturonic acid, tri-, and digalacturonic acid ultimately to galacturonic acid. These 3 enzyme preparations hydrolyzed methyl digalacturonic acid to galacturonic and methyl galacturonic acid (methyl α-D-galactopyranosiduronic acid). Their apparent specificities are qualitatively the same for these substrates under the conditions of these experiments.

Figure 2 shows the reaction course of the tomato and fungal enzyme preparation at 0, 10, 25, 50, 75, and essentially 100% hydrolysis of polygalacturonic acid. When 25% of the bonds were hydrolyzed by both enzyme preparations, galacturonic, di-, tri-, and other oligogalacturonic acids were present. No difference was apparent in the products of partial hydrolysis by either preparation when 50% of the bonds were cleaved. At 70% hydrolysis only galacturonic and digalacturonic acids were observed among the tomato hydrolysis products while both of these uronides and trigalacturonic acid were observed among the products of fungal enzyme hydrolysis. Ultimately only galacturonic acid was present after 168 hours of hydrolysis for the fungal and 500 hours for the tomato enzyme preparations.

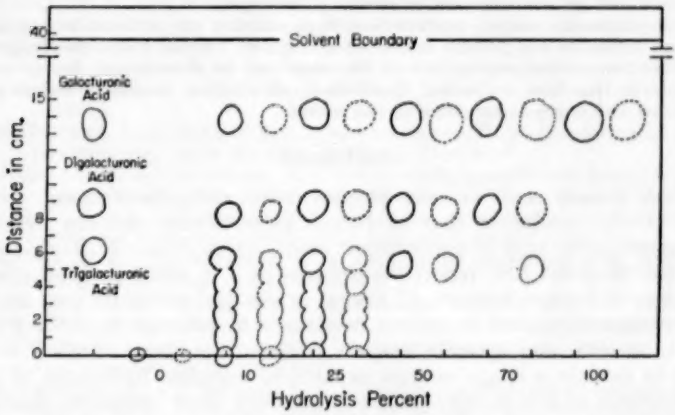

Figure 2. Tracing of a paper chromatogram showing the course of hydrolysis of polygalacturonic acid with tomato enzyme (circles) and fungal polygalacturonase (dotted circles).

The course of the hydrolysis of commercial sodium pectate (containing araban) by a commercial enzyme (Pectinol 100D), fungal (PG), tomato and avocado enzyme preparations is shown in Figure 3. Pectinol 100D produced galacturonic, di-, and trigalacturonic acid and a trace of arabinose at 50% hydrolysis. After complete hydrolysis (about 120% based upon total reducing groups present as compared with anhydrouronic acid content) arabinose and galacturonic acid were present. Fungal enzyme (PG) produced galacturonic, di-, and trigalacturonic acid at 50% hydrolysis. After complete hydrolysis (based upon the anhydrouronic acid content) galacturonic acid and a faint trace of arabinose were noted among the products. Both the tomato and avocado enzyme produced galacturonic, di-, and trigalacturonic acid at 50%

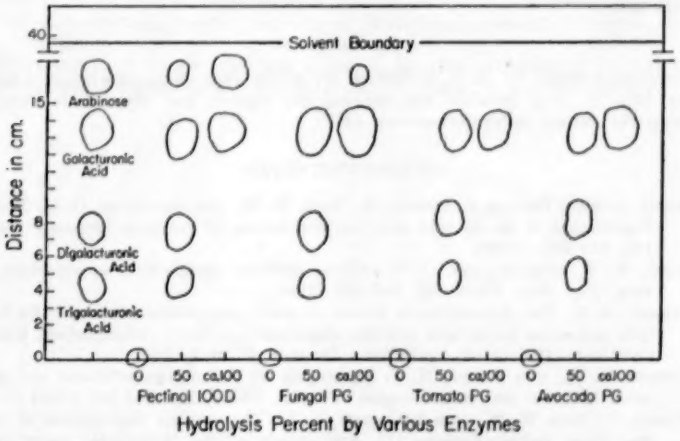

Figure 3. Tracing of a paper chromatogram showing the course of hydrolysis of commercial sodium pectate with pectinol 100D, purified fungal, tomato, and avocado polygalacturonase.

hydrolysis and galacturonic acid at complete hydrolysis.

The commercial enzyme mixture used here contains (in addition to polygalacturonase) arabanase and perhaps several other enzymes. Purified fungal polygalacturonase (PG) has retained only a trace of arabanase and the avocado and tomato enzyme seems to be free from arabanase. Qualitatively all of these enzymes hydrolyze pectic substances and oligogalacturonides in like manner.

DISCUSSION

Both tomato and avocado enzyme have polygalacturonase activity qualitatively similar to that of fungal preparations and can hydrolyze polygalacturonic acid to galacturonic acid. Ayers *et al. (1)*, Dingle *et al. (5)*, and Schubert *(20)* report the presence of 2, 3, and 4 pectin-degrading enzymes in culture extracts of fungal origin and postulate that at least 2 enzymes are required to convert pectic acid to galacturonic acid. Fungal (PG), tomato, and avocado enzyme preparations were assumed in this work to contain a single enzyme capable of complete hydrolysis of polygalacturonic acid to galacturonic acid although there exists the possibility that more than a single enzyme is present in these preparations. There seems to be no justification for postulating the presence of more than a single polygalacturonide-splitting enzyme in the preparations used in this study.

With the exception of tomatoes *(11)* fruits have not been thought to contain polygalacturonase. One reason for this may be the fact that inhibitors, such as that reported in pears *(22, 23)*, occur widely in fruits. Enzymes may develop with ripening and thus occur in measurable quantities only in ripe fruits *(14)*. Weurman *(24)* has shown that pectinase can be demonstrated in a very short period during the ripening process, that is, when the pears were considered "ideal ripe." Future researches may reveal the fact that all pectin-containing plants have enzymes present that hydrolyze polygalacturonides, under certain circumstances, to their constituent units.

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